

PREDATOR-PREY INTERACTIONS INVOLVING THE
SOYBEAN APHID (HEMIPTERA: APHIDIDAE) IN MISSOURI

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by
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CHAPTER I

INTRODUCTION

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was first discovered in the United States in 2000 and has continued to spread throughout the soybean (*Glycine max* L.) growing regions of the Midwest (Venette and Ragsdale 2004). Soybean aphid has caused significant yield losses in northern soybean producing states including Illinois (NSRL 2001), Iowa (Rice et al. 2004), and Minnesota (MacRae and Glogoza 2005). Despite the presence of soybean aphid in southern soybean producing states such as Missouri, yield losses have been limited. Some speculate that soybean aphid rarely reaches economic threshold in Missouri because high summer temperatures negatively affect aphid development. However, this speculation was not supported by preliminary research, as soybean aphid reached outbreak levels in exclusion cages in central Missouri during the summers of 2001 and 2002 (T. L. Clark, personal communication). Within a three week period, soybean aphid populations increased from 5-10 per plant to more than 5,000 per plant (T. L. Clark, personal communication). These data suggest that temperature was not the primary reason soybean aphid populations remain low in Missouri. These data are supported by a climatic study conducted by Venette and Ragsdale (2004) which indicated that southern Missouri should provide a suitable climate for soybean aphid.

Because the pest is exotic, it is unlikely that specialist predators and parasitoids of soybean aphid are present in Missouri. However, native generalist predators, such as the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) and several coccinellid beetle species (Coleoptera: Coccinellidae) may provide effective suppression

of soybean aphid (Bickenstaff and Huggans 1962, Brown et al. 2003, Ellingson and Hogg 2003, Fox and Landis 2003, Koch 2003, Fox et al. 2004, Rutledge et al. 2004, Rutledge and O'Neil 2005). The insidious flower bug is the most common predaceous insect in Missouri soybean (Barry 1973, Marston et al. 1979) and may be responsible for suppressing soybean aphid populations below economic levels. The purpose of this research was 1) to evaluate the native predator complex inhabiting central Missouri soybean fields and to determine their impact on soybean aphid populations and 2) to evaluate insecticide-induced disruption of the predator complex on soybean aphid populations.

CHAPTER II

LITERATURE REVIEW

Origin and Biology. Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to eastern Asia and occurs throughout China, Japan, Malaysia, Philippines, Taiwan and Thailand (Raychaudhuri 1980a). Venette and Ragsdale (2004) report that soybean aphid likely entered the United States in cargo or baggage originating from an indigenous country and that it will likely spread to all soybean (*Glycine max* L.) producing areas in the United States. Soybean aphid was first detected in the United States in 2000 (Wedberg 2000) where it was initially found in Wisconsin followed by Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio and West Virginia. In 2001, soybean aphid was detected in New York, North Dakota, Pennsylvania, South Dakota, and Virginia. In 2002, soybean aphid spread to Delaware, Georgia, Kansas, Mississippi, and Nebraska. In 2003, soybean aphid was detected in Tennessee (Venette and Ragsdale 2004). Soybean aphid was first observed in Missouri during the 2000 growing season where it was detected in 19 counties and since that time has been confirmed in all 65 Missouri soybean-growing counties (T. L. Clark, personal communication).

In Minnesota, Venette (2002) estimated that soybean aphid may expand its range 5-10 km per day. However, the rate of range expansion is not constant throughout the year. In mid to late June soybean aphid spreads slowly (zero to 8 km per day). In late June to early July the spread was rapid at about 13 km a day. During most of July, the spread was moderate at 0-6 km per day. In early August, the spread increased to 10 km a

day. From mid August to the end of the growing season, the rate declined to a moderate to slow pace.

Morphology. The soybean aphid is small, light yellow in color with black cornicles. Alates are winged and have black bodies. Raychaudhuri (1980b) reported that apterae soybean aphid in mm are: body length 1.32, body width 0.73; antennae length 1.04, segment III 0.22, segment IV 0.15, segment V 0.17, segment VI 0.11+0.26; ultimate rostral segment 0.10; second segment of hind tarsus 0.08; siphunculus 0.21; cauda 0.20. Takahashi and Kawakami (1993) discuss soybean aphid morphology in more depth. Typically, soybean aphid has four instars and winged nymphs are determinable in the third instar (Wang et al. 1962, Zhang 1988, Hirano 1996).

Host Plants. In the Midwest, the soybean aphid has a heteroecious (passing through the different stages in the life cycle on alternate and often unrelated hosts) holocyclic lifecycle (Ragsdale et al. 2004); this entails alternating hosts and sexual reproduction during parts of its lifecycle. Soybean aphid utilizes primary hosts during the fall and winter and secondary host during the summer. This is similar to lifecycles observed in China and Japan with the exception of the primary and secondary host plant species (Ragsdale et al. 2004). In China and Japan, primary hosts are dahurian buckthorn, *Rhamnus davurica* Pallus (Rhamnales: Rhamnaceae) (Hirano 1996), and Japanese buckthorn, *Rhamnus japonica* Maxim. (Rhamnales: Rhamnaceae) (Takahashi and Kawakami 1993). There are three confirmed primary hosts of soybean aphid in the Midwest: common buckthorn, *Rhamnus cathartica* L. (Rhamnales: Rhamnaceae), alderleaf buckthorn, *Rhamnus alnifolia* L'Heritier (Rhamnales: Rhamnaceae) and

lanceleaf buckthorn, *Rhamnus lanceolate* Pursh (Rhamnales: Rhamnaceae) (Voegtlin et al. 2004, 2005, Yoo et al. 2005).

Secondary hosts in Asia include; wild soybean, *Glycine soja* Sieb & Zucc. (Rosales: Fabaceae), cultivated soybean, *Glycine max* (L.) Merr. (Rosales: Fabaceae), kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Rosales: Fabaceae), in Korea and *Pueraria javanica* (Roxb.) Benth (Rosales: Fabaceae) in the Philippines (Wang et al. 1962, Hirano 1996). In the Midwest, Alleman et al. (2002) found red clover, *Trifolium pratense* L. (Rosales: Fabaceae), to be an excellent secondary host of soybean aphid in the laboratory; however, it does not appear to play a significant role in spring colonization of soybean (Ragsdale et al. 2004). Crimson clover, *Trifolium incarnatum* L. (Rosales: Fabaceae), was also an excellent secondary host of soybean aphid. Berseem clover, *Trifolium alexandrinum* L. (Rosales: Fabaceae), and kura clover, *Trifolium ambiguum* M. Beib. (Rosales: Fabaceae), are less suitable secondary hosts. White clover, *Trifolium repens* L. (Rosales: Fabaceae), white sweet clover, *Melilotus alba* Medikus (Rosales: Fabaceae), and yellow sweet clover, *Melilotus officinalis* (L.) (Rosales: Fabaceae) Lam., are extremely poor secondary hosts of soybean aphid (Alleman et al. 2002).

Life Cycle. In the Midwest, soybean aphid over-winters in the egg stage on *Rhamnus* spp. (Ragsdale et al. 2004). Eggs hatch in the spring and develop into wingless (apterae) fundatrices. Fundatrices then produce a second generation of primarily wingless females. The third and subsequent generations are primarily winged morphs (alates), which migrate to soybean (Ragsdale et al. 2004). Liu et al. (2004) observed a time lag between colonization and population growth and suggest this is the time it takes

alates to arrive, produce nymphs, and for the progeny to become reproductive. More than 15 generations may occur on soybean (Wang et al. 1962). Li et al. (2000) report that 18-22 generations are possible each summer in China's Jinan province. In a three year study conducted in Iowa, Lang (2003) found that soybean fields colonized in early or late June would exhibit two soybean aphid population peaks, one in late July and another in Mid-August. Soybean fields colonized later, in early July, would likely exhibit only one peak in Mid-August (Lang 2003).

Li-hua and Rui-lu (1993) examined factors affecting the production of alate soybean aphids. They determined that crowding of apterous adults promotes an increase in alate populations. Conversely, crowding of nymphs and alate adults does not enhance alate populations. They also observed that host plant quality affects the production of alates. For example, soybean aphid on young tender leaves produced less alates than those on mature leaves. Temperature also affected the production of alates; more alates were produced at lower temperatures (21°C) and fewer alates were produced at higher temperatures (25 and 31°C) (Li-hua and Rui-lu 1993).

In autumn, winged females (gynoparae) develop on soybeans and migrate to *Rhamnus* spp. where they feed and produce nymphs that develop into oviparae. Winged males, also developing on soybean in autumn, migrate to *Rhamnus* spp. in search of the oviparae. The males and oviparae mate and deposit eggs on *Rhamnus* spp., which occurs from late October to mid November (Ragsdale et al. 2004). They found the most common oviposition site to be the bud axil, although eggs could be deposited anywhere on the tree.

Development Factors

Hirano et al. (1996) and McCornack et al. (2004) have examined the role of temperature on soybean aphid developmental rate. Developmental time from first instar to adult took 5.2 days at 22°C and 3.9 days at 27°C (Hirano et al. 1996). At temperatures up to 27°C, the developmental time to adult decreases with increasing temperature (Hirano et al. 1996). The converse is true beyond 27°C as developmental time increases and survival rate decreases at 32°C. McCornack et al. (2004) observed that adults held at 20°, 25°, 30°, and 35°C produced nymphs within 24 hours. Nymphs held at 35°C died within 11 days without producing offspring while nymphs produced at 20°, 25°, and 30°C completed development. McCornack et al. (2004) found no significant differences in pre-reproductive times (time from emergence until sexual maturity) at temperatures of 20°, 25°, and 30°C. Hirano et al. (1996) observed that mean reproductive period, lifetime fecundity, and adult longevity were significantly greater at 22°C. Regression analysis of this data showed the time from nymph to adult to be 67.1 degree days for soybean aphid, with a minimum development threshold of 9.5°C (Hirano et al. 1996).

Population Growth Factors. At 22°C, the finite rate of increase (λ) was 1.225-1.897 [λ is a proportional change in population size and has no units] while the intrinsic rate of increase (r) was 0.445 [individuals/(individuals*time)] (Hirano et al. 1996). An intrinsic rate of increase greater than 0 indicates the populations is increasing exponentially. At 20°, 25°, and 30°C the intrinsic rate of increase was 0.368, 0.474, and 0.375 [individuals/(individuals*time)], respectively (McCornack et al. 2004). At 22° and 27°C, the intrinsic rate of increase was 0.45 and 0.53, respectively (Hirano 1996).

Soybean aphid reared at 35°C had an intrinsic rate of increase of -0.383 (McCornack et al. 2004). Su et al. (1996) reported that when $\lambda < 2$, aggregation results from environmental factors. When $\lambda > 2$, aggregation results from a combination of insect behavior and environmental factors. In all cases, as temperature increased above 30°C, mortality increased and longevity decreased (McCornack et al. 2004). Mean fecundity at the time of peak production at 20°, 25°, and 30°C were 6.5, 9.5, and 3.5 nymphs per day (McCornack et al. 2004). Hirano et al. (1996) found the lower developmental threshold (K_1) to be 9.5°C, while McCornack et al. (2004) found K_1 to be 5.6°C. Upon combining the data sets, K_1 value was 8.6°C (McCornack et al. 2004).

Temperature and Precipitation. When investigating population prediction methods, Zongdai and Jifeng (1990) reported a significant correlation between temperature, precipitation, and the occurrence of soybean aphid in Jilin Province, Japan. They observed that temperatures between 22.3° and 23.4°C with precipitation $< 20\text{mm}$ were favorable for soybean aphid population growth, while temperatures between 20° and 21°C with precipitation $> 55.1\text{mm}$ were not favorable for soybean aphid population growth. In China, Tian et al. (1990) reported that conditions of intermediate humidity and higher temperatures were best for soybean aphid population growth. Together, these studies indicate that higher temperatures (22°C to 30°C), lower precipitation and lower humidity are best for soybean aphid population growth in East Asia.

Plant Phenology. Plant phenology impacts soybean aphid development as Van Den Berg et al. (1997) found that the net reproductive rate was higher on 3 week old plants than on 7 week old plants. On 3 week old plants, all aphids survived to

reproduction with 50% mortality by day 15. On 7 week old plants, 30% of the aphids died before becoming reproductive with 50% mortality by day 12.

Plant Nutrition. In a two year study conducted in Wisconsin, Myers et al. (2005b) determined in laboratory studies that plant potassium (K) level affected soybean aphid development. They found that soybean aphid grown on K deficient soybean leaves had a 39% higher net reproductive rate, 15% higher intrinsic rate of increase, and a 6% higher finite rate of increase than soybean aphid grown on healthy soybean leaves. Mean generation time did not differ between the two populations. However, they found that this did not translate into higher soybean aphid populations in soybean fields demonstrating K deficiencies. While both K deficiency and soybean aphid feeding were capable of reducing soybean yield, they did not act synergistically in reducing yield. Soybean aphid feeding reduced yield by \approx 19%, while growing soybean in K deficient soil decreased yield by an additional 32%.

Hu et al. (1992) determined that the nitrogen (N) content of soybean leaves affected soybean aphid populations in China. Soybean aphid populations increased as N content in leaves increased and decreased as N content in leaves decreased. These results were not affected by soybean variety.

Another study conducted by Qi et al. (1993) determined that the lignin level of soybean leaves affected soybean aphid populations in China. Higher levels of lignin resulted in lower populations of soybean aphid, while lower levels of lignin resulted in higher populations of soybean aphid.

Within-Plant Distribution. Soybean aphids are found on different parts of the soybean plant at different phenologies. During actively growing vegetative stages,

soybean aphids are found on stems and young leaves. During the reproductive stages, soybean aphids are found on the underside of leaves, stems, and pods (Ragsdale et al. 2004).

Ito (1953) determined that soybean aphid colonization varied depending on the number of plants available. On a single soybean plant, Ito observed that soybean aphid colonization would begin on young leaves, reproduction would occur until saturation population densities were reached, and then soybean aphid would move to older leaves which had not yet been infested. When multiple soybean plants were present, Ito observed that soybean aphid colonization would also begin on young leaves. However, when saturation densities were reached, soybean aphid would move to young leaves of the other soybean plants. Only when all young leaves had reached saturation population densities would soybean aphids begin to colonize older leaves. Ito determined the saturation density population to be 250 aphids per 10 cm² leaf area.

Spatial Distribution. The spatial distribution of soybean aphid populations within a field changes over time. Ragsdale et al. (2004) observed that in many North Central United States, early season soybean aphid populations were aggregated. Colonies were small and consisted of early instars with no adults. They suggest this is the result of winged migrants feeding for a short time and depositing nymphs, then moving to another host plant. This behavior resulted in soybean fields being colonized rapidly with no edge effect in early spring. In contrast, fields colonized in mid-summer may have a distinct edge effect. Ragsdale et al. (2004) proposed that fields which remain aphid free until July, like many in the North Central United States, are likely being colonized by soybean aphid migrating from other soybean and not from the over-

wintering host. This is consistent with present views on the spread of soybean aphid adults in the North Central United States. It is believed that soybean aphid emerges, migrates to soybean, and produces alates which are then carried by winds to infest soybean downwind.

Sampling. Several sampling methods have been described in recent years in the North Central United States. These include whole plant counts, enumerative sampling, binomial sampling, and rating scales.

Onstad et al. (2005) determined that whole plant counts of 50 plants per field, within 50 m from the field edge, should be completed for the most reliable estimate of the population. However, they found this to be very time consuming at high soybean aphid densities and when the canopy was closed.

In a three year study conducted in Minnesota, Hodgson et al. (2004) developed enumerative and binomial sequential sampling plans for soybean aphid. Enumerative sampling provides the most accurate estimation of soybean aphid populations while binomial sampling is more suitable for making pest management decisions. Enumerative sampling counts the actual number of aphids on a plant. As soybean aphid densities increase the number of plants that must be sampled decreases; as soybean aphid densities decrease, the number of plants that must be sampled increases. In contrast, binomial sampling is a tally system in which any plant with greater than 40 soybean aphids is considered infested. This results in fewer sample units and thus less time invested in sampling. (Hodgson et al. 2004)

Hodgson et al. (2004) determined the most effective sampling plan, in terms of reducing incorrect no treatment decisions, was a tally threshold of 40, mean density of

250 aphids, with 84% of plants infested. Initial sampling consists of 11 plants. The number of infested (≥ 40 aphids) and non-infested (< 40 aphids) plants are counted. If the result falls within the treat or no-treat zone, sampling stops; if the result falls in the middle, sampling continues. Hodgson et al. (2004) recommend sampling only up to 31 plants to reach a decision. If no decision is reached, resample in three to four days.

DiFonzo and Hines (2002) developed a rating scale for soybean aphid sampling. Leaflets are rated on a scale of 0-4, based on soybean aphid density. 0 having no aphids, 1 having 1-10 aphids, 2 having 11-25 aphids, 3 having 26-99 aphids, and 4 with more than 100 aphids. Treatment would be recommended if average leaf ratings were 3 or more. Rating scales are quick and easy and are most useful for evaluating fields before and after spraying.

Soybean Growth Stages. Treatment for soybean aphid is dependent on the growth stage of the plant at the time of infestation. Soybean plants begin in the vegetative stages (V) and proceeds through the reproductive stages (R) (Fig. 1). A plant in the VE stage has just emerged from the soil. During the VC stage, the cotyledons have separated and the first unifoliate leaves are beginning to unfold. During V1, unifoliate leaves are fully expanded and the first trifoliate (a trifoliate is also referred to as node) is present. During V2, a second node is present. This continues the rest of the season with each new trifoliate. Once a plant reaches reproduction, it is described using a V and R. Plants in R1 and R2 are in beginning bloom and full bloom, respectively. Plants in R3 and R4 are in beginning pod and full pod, respectively. R5 and R6 are in beginning seed and full seed. R7 and R8 are beginning maturity and full maturity. To determine the growth stage of a field, several plants should be examined and an average determined.

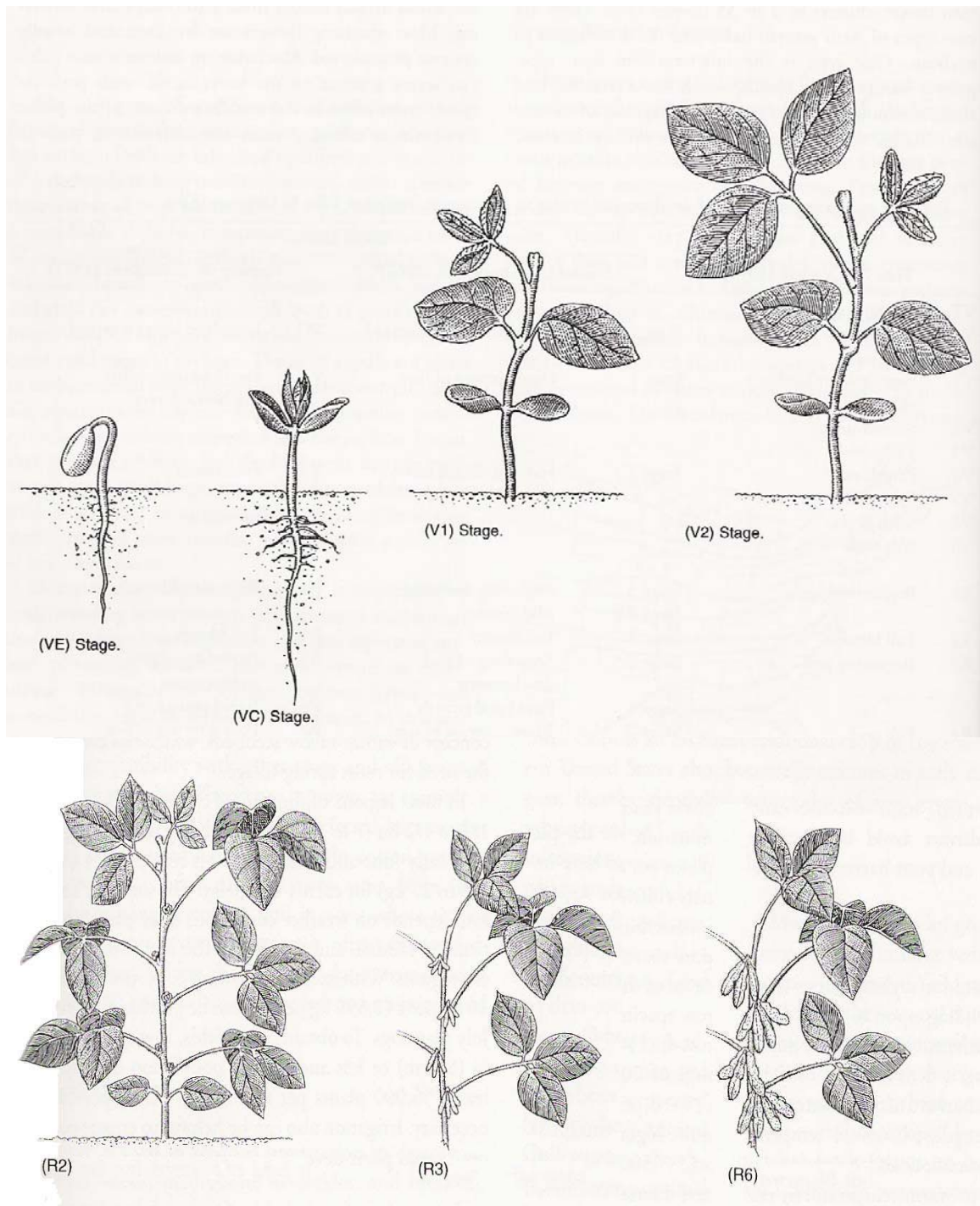


Fig. 1. Soybean growth stages as described by Fehr and Caviness (1977) for indeterminate varieties. VE-emergence; VC-cotyledon expanded; V1-first node; V2-second node; R2-full bloom; R3-beginning pod; R6-full seed. Reproduced from Higley and Boethel (1994).

Threshold. Economic thresholds for soybean aphid are still being debated. However, Sloderbeck et al. (2005) recommend an economic threshold of 50 soybean aphids per leaflet in pre-reproductive plants and an economic threshold of 250 soybean aphids per plant in reproductive plants (through pod set). At R3 they recommend an economic threshold of 1000 soybean aphids per plant and for R4-R5 they recommend 1500 soybean aphids per plant.

Venette et al. (2004) devised a soybean aphid population growth estimator which is available online <http://www.soybeans.umn.edu/files/Soybean%20aphid%20growth%20estimator%20v1-2.xls>. Days until economic threshold and doubling time are calculated using local weather forecasts (minimum and maximum temperature) and initial soybean aphid numbers. Estimates are based on the maximum rate of population growth, although other factors such as predators and plant quality may keep soybean aphid populations from growing as quickly (Venette et al. 2004).

Myers et al. (2005a) conducted studies over a three year period to determine the optimal timing of insecticide applications for suppression of soybean aphid. They concluded that when soybean aphid numbers are high, insecticide applications at R2 and R3 were most effective at preventing yield loss. However, they stress that soybean aphid populations vary among years and planting dates and so insecticide application may not always be necessary. Myers and Wedberg (2002) investigated the optimal planting time to reduce soybean aphid populations in Wisconsin. They concluded that early planted soybean, while they may be susceptible to initial infestation, were less susceptible when soybean aphid populations were high. They also determined that one well timed spray at

the beginning of the peak aphid population (when the majority of the plants have soybean aphid numbers greater than 200) would prevent yield losses.

Insecticide resistance has already been reported in China where Huang et al. (1998) report decreased control effects of organophosphorus insecticides such as dimethoate or omethoate against soybean aphid. However, an average of 91-95% control effects was achieved using imidacloprid at rates of 22.5 g a.i./hectare to 45 g a.i./hectare. As an alternative to chemical insecticide applications, Dai and Zu (1997) investigated the toxicity of a mixture containing Gray-green mold metabolites and plant extracts, called G-P compound bioinsecticide on soybean aphid. The gray-green mold which provided activity was identified as *Paecilomyces griseoviride* Onions & G.L. Barron (Eurotiales: Trichocomaceae) strain U-2, and was originally isolated from dead infected cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Dai 1997). The mixture resulted in 89.5 and 91.0% soybean aphid mortality, 48 and 96 hours after application, respectively, which were comparable to those achieved by a chemical insecticide, omethoate. G-P bioinsecticide demonstrated significantly less toxicity to natural enemies than did omethoate in both laboratory and field studies (Dai and Zu 1997).

Economic Significance

Direct Yield Impacts. Soybean aphid affects yield directly through feeding (removal of photosynthates and reduction of photosynthesis) and indirectly through transmission of viruses. Removal of photosynthates can be exacerbated by severe environmental conditions. For example, Fischer and Fanta (2003) speculated that under

drought conditions plants are less able to compensate for the loss of moisture caused by soybean aphid, resulting in enhanced susceptibility to infestation. Soybean aphid infestations may also cause leaf yellowing and curling, plant stunting, poor canopy development, and reduced yields (Catangui 2002b). Honeydew, secreted by soybean aphids, can lead to sooty mold, in which leaves look sticky or sooty (Catangui 2002b).

Phenological timing of infestation is an important factor influencing yield loss due to soybean aphid. Yield is most affected when soybean aphid feeding occurs during the V2 stage of growth where losses from 2.7 to 51.8% have been reported (Wang et al. 1996). Reduction in yield may be the result of reduced photosynthetic rates in infested leaves; photosynthetic rates were significantly lower in infested leaves than uninfested leaves, even at low infestation levels (20 aphids per leaflet) (Macedo et al. 2003). Dai and Fan (1991) determined that younger plants can compensate for low infestations but are sensitive to high infestations, while later phenologies can tolerate higher numbers without significant reduction in yield. In China, Wang et al. (1994) found that the number of soybean aphids per plant was negatively correlated with plant height, number of pods, and number of seeds per plant.

Virus Transmission. Prior to the introduction of soybean aphid, virus transmission in soybean fields was most likely due to transient, non-colonizing aphids or other insect species (Clark and Perry 2002). This has the potential to change as soybean aphid is a vector of several viruses that impact soybean. Soybean aphid is a confirmed vector of soybean mosaic virus (SMV) (Yongxuan et al. 1988, Hill et al. 2001, Alleman et al. 2002, Wang and Ghabrial 2002, Domier et al. 2003), alfalfa mosaic virus (AMV) (Hill et al. 2001, Alleman et al. 2002), cucumber mosaic virus (CMV) (Alleman et al.

2002), potato virus Y (PVY) (Davis et al. 2005), and soybean dwarf virus (SDV) (Hirano 1996). Clark and Perry (2002) and Domier et al. (2003) confirmed that multiple isolates of SMV and AMV, both field and laboratory reared, are transmitted by soybean aphid .

Soybean aphid transmits PVY at transmission rates ranging from 14-75% (Davis et al. 2005), SMV is transmitted at an average transmission rate of 35% (Wang and Ghabrial 2002). Transmission was most efficient when the soybean aphid briefly probed the infected soybean plant before moving to a healthy soybean plant (Wang and Ghabrial 2002). Soybean aphid was found to transmit AMV from red clover to soybean at low efficiency levels (Alleman et al. 2002). Piñeyro et al. (2002) determined that soybean aphid could not transmit AMV to *T. ambiguum* (a relative of red clover), though *T. ambiguum* is a host plant. Wang and Ghabrial (2002) speculate that soybean aphid also transmits bean yellow mosaic virus (BYMV), peanut mottle virus (PeMoV), and peanut stunt virus (PSV) but this has not been documented. Soybean dwarf virus, vectored by several aphid species, has been reported for the first time in soybean in Wisconsin; while the vector has not been determined, 100% of plants were infested with soybean aphid (Phibbs and Barta 2004).

Natural Enemies of Soybean Aphid

Observations from Asia indicate that soybean aphid populations were extremely low in environments similar to the North Central United States (Fox et al. 2004). Soybean aphid populations in Asia are believed to be under the control of a number of

natural enemies (Ma et al. 1986, Chang et al. 1994, Rongcai et al. 1994, Han 1997, Van Den Berg et al. 1997, Liu et al. 2004).

Pathogens of Soybean Aphid. Little research into fungal pathogens of soybean aphid has been performed. Most available data is the result of a recent study by Nielsen and Hajek (2005). Nielsen and Hajek (2005) identified six entomophthoralean fungal species attacking soybean aphid. These were: *Pandora neoaphidis* (Remaud. et Henn.) Humber (Entomophthorales: Entomophthoraceae), *Pandora* sp., *Conidiobolus thromboides* Drechsler (Entomophthorales: Ancylistaceae), *Entomophthora chromaphidis* (O.F. Burger & Swain) (Entomophthorales: Entomophthoraceae), *Zoophthora occidentalis* (Thaxter) Batko (Entomophthorales: Entomophthoraceae), and *Neozygites fresenii* (Now.) Remaud. et Keller (Entomophthorales: Neozygitaceae). Of these, *P. neoaphidis* was the most abundant, causing 84% infection during a soybean aphid outbreak in 2003. Nielsen and Hajek (2005) found that fungal infections were found early in the season and were strongly associated with soybean aphid density. Levels of infection were associated with subsequent declines in soybean aphid populations. The lethal time (from infection to death) for soybean aphid has not been determined; however, other aphid species lethal times for *P. neoaphidis* vary from 3.5 to 5.1 d at 20°C. While early season infections were capable of greatly reducing soybean aphid populations, late season infections seemed less able to affect populations. However, late season infections may be more important in the long term suppression of soybean aphid populations through the development of a reservoir of inoculum to infect the next spring. Alate aphids were 200-400% more susceptible to infection than other morphs. Once alates became infected, they could spread the infection as they were still

capable of long flights. Nielsen and Hajek (2005) conclude that existing fungi play an important role in suppressing soybean aphid populations in soybean in New York State from bloom until pods are mature.

Parasitoids of Soybean Aphid. Because soybean aphid is native to Asia, most research has been performed in these areas. In Korea, several primary parasitoids have been found attacking soybean aphid (Chang et al. 1994). The common primary parasitoids found to be effective against soybean aphid include *Aphidius cingulatus* Ruthe (Hymenoptera: Braconidae), *Ephedrus persicae* Froggat (Hymenoptera: Braconidae), and *Ephedrus plagiator* (Nees) (Hymenoptera: Braconidae) (Chang et al. 1994). *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Aphidiidae), *Aphidius salicis* Haliday (Hymenoptera: Braconidae), *Aphidius absinthii* Marshall (Hymenoptera: Braconidae), *Ephedrus validrus* Haliday (Hymenoptera: Braconidae), and *Lypolexis gracillis* Förster (Hymenoptera: Aphidiidae) were all primary parasitoids accounting for less than 10% occurrence. Primary parasitoids represented 27.1% of mummies collected in the field (Chang et al. 1994).

In China, *L. japonica* is the dominant parasitoid of the soybean aphid with parasitism rates from 10.3-52.6% found in the field (Gao 1994). Hirano (1996) found parasitism rates of soybean aphid averaging 56% with a maximum of 76% for *L. japonica* in China. Gao (1994) found that *L. japonica* could suppress soybean aphid early in the season and thus decrease populations later in the season.

While native parasitoids of soybean aphid are common in Asia, few parasitoids have been identified in North America (Fox and Landis 2003, Rutledge et al. 2004). Nielsen and Hajek (2005) recently discovered three species of parasitoids attacking

soybean aphid in North America; however, parasitism rates were low (7-15%) with high nonemergence rates (55.9%). Ellingson and Hogg (2003) released an exotic soybean aphid parasitoid, *Aphelinus albipodus* (Hayat and Fatima) (Hymenoptera: Aphelinidae), in Wisconsin. Following the introduction, 12% parasitism was observed within one week.

Predators of Soybean Aphid. Several predatory insects have been reported feeding on soybean aphid in Asia. Ma et al. (1986) provides an overview of soybean aphid predators with original citations therein. Predators reported upon include: the seven spotted lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Wang et al. 1991); *Adonia variegata* Goeze (Coleoptera: Coccinellidae) (Wang et al. 1991); the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Wang et al. 1991); *Propylaea japonica* (Thunberg) (Coleoptera: Coccinellidae) (Wang et al. 1991, Han 1997); *Coelophore saucia* Mulsant (Hemiptera: Pentatomidae) (Dai 1990, Han 1997), and *Scymnus hoffmanni* Weise (Coleoptera: Coccinellidae) (Han 1997) in China.

Rutledge et al. (2004) in a two year study identified the insects most likely to contribute to soybean aphid suppression in Indiana. The most numerous predators were the insidious flower bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae), and *H. axyridis* which together accounted for over 85% of all predators found. Based on the seasonal occurrence of the predators and their abundance in the field, Rutledge et al. (2004) hypothesized that insects occurring early in the season and in high numbers are more likely to prevent outbreaks. They concluded that key natural enemies include *O. insidiosus*, *H. axyridis*, and to a lesser extent *C. septempunctata* and the convergent lady

beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae). They further concluded that predators can significantly reduce soybean aphid establishment and that the role of predators becomes more important the later aphid immigration occurs.

Efficacy of Selected Soybean Aphid Predators. Two coccinellid species and one hemiptera have been studied as predators of soybean aphid. Lin et al. (1999) investigated the role of *H. axyridis* as a predator of the soybean aphid in China. Ellingson and Hogg (2003) determined that in Wisconsin, a rate of one *H. axyridis* larvae per seven plants was capable of suppressing soybean aphid population growth.

Brown et al. (2003) investigated the potential of another ladybeetle, *Scymnus louisianae* Chapin (Coleoptera: Coccinellidae), for control of soybean aphid in Kentucky. They determined that first thru fourth instars of *S. louisianae* consumed an average of 3, 8, 24, and 63 soybean aphids, respectively, during each stage. During their adult lifetime, female and male *S. louisianae* beetles consumed an average of 1261 and 665 soybean aphids, respectively. Brown et al. (2003) conclude that when *S. louisianae* is reared on a diet exclusively of soybean aphid, which it has no prior relationship with, its developmental time, survivorship, oviposition rates, and predation rates remain normal. This suggests that *S. louisianae* could be useful as a biological control agent.

Rutledge and O'Neil (2005) investigated the effectiveness of *O. insidiosus* as a predator of the soybean aphid. In a study conducted in Japan by Oku and Kobayashi (1966), when both soybean aphid and *N. variabilis* were present in high numbers, *Orius* sp. preyed more upon the soybean aphid because it is not highly mobile and tends to form clusters.

Several exclusion studies have been conducted to evaluate the role of predators in establishment and spread of soybean aphid. Fox et al. (2004) found that foliar-foraging predators prevented soybean aphid population growth and maintained numbers below 10 aphids per plant, while soybean aphid populations in exclusion cages exceeded 200 aphids per plant. When predators were excluded (in previously open cages), small soybean aphid populations increased. They concluded that predators kept soybean aphid populations low in the previously open cages. *Orius insidiosus* adults constituted 61.7% and 55.2% of predators observed in open and frame cages, respectively. When predators had access to previously high soybean aphid populations, soybean aphid populations decreased while predator abundance increased from less than 5 to over 60 predators per m². *Harmonia axyridis* adults, *O. insidiosus* adults, and *Leucopis* spp. (Diptera: Chamaemyiidae) larvae were the most abundant predators, comprising 22.9, 14.0, and 30.6% of the total predators, respectively. *Harmonia axyridis* populations remained elevated throughout the trial, while *O. insidiosus* and *Leucopis* spp. population increases were temporary. Fox et al. (2004) concluded that not only were predators capable of keeping soybean aphid populations low, but they also quickly reduced high soybean aphid populations.

Before the arrival of the soybean aphid, it was generally accepted that the soybean thrips, *N. variabilis*, was the primary prey species of *O. insidiosus* (Marston et al. 1979). Thrips arrive early in the season (unifoliate stage, VI) in both early and late planted soybean, reproduce rapidly, and are abundant by the time *O. insidiosus* arrives (V5-V8 for May planted; V2-V4 for June planted) (Isenhour and Marston 1981).

Plant Resistance. Because soybean aphid has been present in Asia much longer than in North America, resistant varieties adapted to Asian environmental conditions are already available. Yi-heng (1988), in a multi-year study in China, identified two varieties of resistant soybean. He suggests that due to variability in performance over different years, resistance testing should be carried out over several years. He also suggests that resistance should be investigated under different soybean aphid populations; when soybean aphid populations peak, a week after populations peak, and more than a week later when soybean aphid populations are declining. In China, in an effort to understand the inheritance of resistance to soybean aphid, Sun et al. (1991) crossed susceptible cultivated soybean with resistant wild soybean. Their data suggest two major independent gene pairs and some other minor genes control resistance.

Resistance to soybean aphid in North American cultivars has only recently been investigated by Hill et al. (2004a), who discovered six resistant *G. max* accessions. Resistant *G. max* accessions could serve as a source of resistance when crossed with cultivated soybean (Hill et al. 2004a). In three of the six accessions discovered, it is believed that antibiosis was responsible for the resistance. Hill et al. (2004b) reported resistance in three ancestral soybean cultivars of North America; Dowling, Jackson, and PI 71506. Resistance was also reported in two ancestors of Dowling and Jackson (Palmetto and CNS) and four other germplasm accessions (Sugao Zairai, Sato, T260H, and PI 230977). Resistance in Dowling, Jackson, and Palmetto was attributed to antibiosis while resistance in PI 71506 and CNS was attributed to antixenosis.

Li et al. (2004) investigated the effect of resistance on soybean aphid biology. Soybean aphid grown on resistant varieties had significantly reduced fecundity,

longevity, and percent maturation, with greatly increased mortality. Soybean aphids placed on resistant leaves departed between 8 and 24 h after transfer while those placed on susceptible leaves remained more than 48 h. This suggests that antibiosis and antixenosis play a role in soybean resistance to soybean aphid.

Certain plant volatiles given off by soybean plants infested with soybean aphid aid in plant defense. Zhu and Parks (2005) isolated methyl salicylate from soybean leaves damaged by soybean aphids. They found that antennae of *C. septempunctata* respond significantly to methyl salicylate. Methyl salicylate has also been demonstrated to attract predatory mites, *Phytoseiulus persimilis* Athias-Henriot (Acari:Phytoseiidae), predatory bugs, *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae), the minute pirate bug, *Orius tristicolor* (White) (Hemiptera: Anthocoridae), big eyed bugs, *Geocoris pallens* Stal (Hemiptera: Lygaeidae), and the lady beetle, *Stethorus p. punctatum* (LeConte) (Coleoptera: Coccinellidae) (James 2003).

While resistance to soybean aphid has been detected in several varieties, only Pioneer Hi-Bred International, Inc. is currently marketing commercial soybean varieties as having antibiosis against the soybean aphid. As of the 2006 growing season, Pioneer will offer soybean varieties rated for performance against the soybean aphid (Pioneer Hi-Bred International 2006a). Several varieties displaying exceptional or above average antibiosis ratings against soybean aphid will be available to soybean producers in the Midwest. Since no variety currently available is completely resistant to soybean aphid, Pioneer suggests growers use these ratings as part of a pest management plan when making scouting and insecticide application decisions (Pioneer Hi-Bred International 2006b).

Cultural Control. Chung et al. (1980), in a study conducted in Korea, found that high plant numbers decreased soybean aphid populations. Thus, in areas of severe soybean aphid pressure, planting early with high plant densities using the newly rated aphid resistant seed would be recommended (Gaska et al. 2002, Pioneer Hi-Bred International 2006b).

CHAPTER III

POPULATION GROWTH OF *APHIS GLYCINES* MATSUMURA UNDER VARYING LEVELS OF PREDATOR EXCLUSION

ABSTRACT In Missouri, there is a unique predator complex that suppresses soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), populations prior to reaching economic threshold. Preliminary research has shown that when soybeans were infested with soybean aphids under normal field conditions, populations did not become well established. However, when plants were caged to exclude predators, soybean aphid populations grew rapidly. To determine which predator is most responsible for suppression of soybean aphid populations in Missouri, soybean plants were caged using different size meshes. Three sizes of mesh were used; one which excludes all insects, one which excludes insects larger than thrips and one which excludes insects larger than *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), a principal predator. As a control, cages were placed over soybean without mesh. Caged plants were infested with soybean aphid at the plant growth stages V5, R1, and R3. Fifteen aphids were transferred using a fine camel's hair brush to the uppermost expanded trifoliolate. Soybean aphids used were colony reared and of the same ages (<48 hours). Sampling consisted of weekly visual observations of the caged plants for soybean aphids and aphidophagous predators; in particular, *O. insidiosus* (nymph and adult) and coccinellids (Coleoptera: Coccinellidae) (larvae and adult). Information gained from this study will allow us to assess the key soybean aphid predators at different soybean phenologies.

KEY WORDS predator complex, predator exclusion, *Orius insidiosus*

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive species first discovered in the United States in 2000 (Wedberg 2000). Since initial discovery, it has been detected in 20 states and also parts of Canada (Venette and Ragsdale 2004). Despite the presence of soybean aphid in southern soybean producing states, serious outbreaks have been limited. Some have speculated that soybean aphid rarely reaches the economic threshold in Missouri because high summer temperatures negatively affect soybean aphid development. However, this speculation was not supported by preliminary research, as soybean aphid reached outbreak levels in exclusion cages in central Missouri during the summers of 2001 and 2002 (T. L. Clark, personal communication). Within a three week period, soybean aphid populations increased from 5-10 per plant to more than 5,000 per plant (T. L. Clark, personal communication). These data suggest that temperature was not the primary reason soybean aphid populations remain low in Missouri. These data are supported by a climatic study conducted by Venette and Ragsdale (2004) which indicated that southern Missouri should provide a suitable climate for soybean aphid.

The objective of this study was to examine the effect of native Missouri predators on the newly introduced soybean aphid. More specifically, to elucidate which predators are most responsible for controlling soybean aphid populations in Missouri and also whether they are capable of halting or delaying soybean aphid establishment.

Materials and Methods

Experimental Design. This study was conducted at the University of Missouri, South Farms in the summer of 2004. South Farms (92° 17' W, 92° 12' N; elevation ≈ 893') is located approximately 5.8 km southeast of University of Missouri campus. The experiment was a randomized complete block design in a 4 × 4 (infestation date × mesh size) factorial arrangement with repeated measures and was replicated four times. Cages were 1.5 m apart and replications were 6 m apart within the soybean field. Fields were cultivated using reduced primary tillage (disc), cages were placed and soybean variety DKB 38-52 (Monsanto Company, St. Louis, MO) was planted six seeds to a cage on 22 June 2004. Cages and nearby plots were kept weed free by the application of Roundup WeatherMAX® at a rate of 864 g (AI)/ha (Monsanto Company, St. Louis, MO) on 17 July and 13 August.

Predator Exclusion Trials. Aphidophagous predators (Coccinellidae, Syrphidae, Chrysopidae, and Anthocoridae) and soybean aphid densities were monitored throughout the season in cages with three different sizes of mesh and a control cage with no mesh. Cage frames were constructed of PVC pipe and fittings (1.3 cm outside diameter; Lasco Fittings, Inc., Brownsville, TN). Cages were 1 m³ with approximately 10 cm placed in the soil and secured with 10 cm wire landscape staples (Fig. 2). Three sizes of mesh were used: Econet S (300 squares per cm), Econet L (140 squares per cm) (LS Americas, Charlotte, NC) and mosquito netting (30 squares per cm) (Barre Army Navy Store, Barre, VT). Mesh was sewn to fit the cage frame with excess material on the bottom to allow the mesh to be buried. Mesh was buried in the soil and secured with 10 cm wire

landscape staples. Access was provided by Velcro® closures along the top and side of one panel. Control cages consisted of just the PVC frame with no mesh. Control cages were designed to allow full access to all insects.

Mesh was chosen based on predator size. Small mesh was meant to exclude all arthropods, even mites. Medium mesh was meant to exclude all insects larger than thrips and whiteflies. Large mesh was meant to exclude all insects larger than *O. insidiosus*. However; in the exclusion cages with mesh (small, medium, and large), predators which should have been excluded were sometimes present. This occurred because the adult insects, particularly Coccinellidae, Chrysopidae and Syrphidae, would lay eggs on the outside of the mesh and the larvae would crawl thru. When this occurred, the number of insects was recorded and the insects removed from the cage.

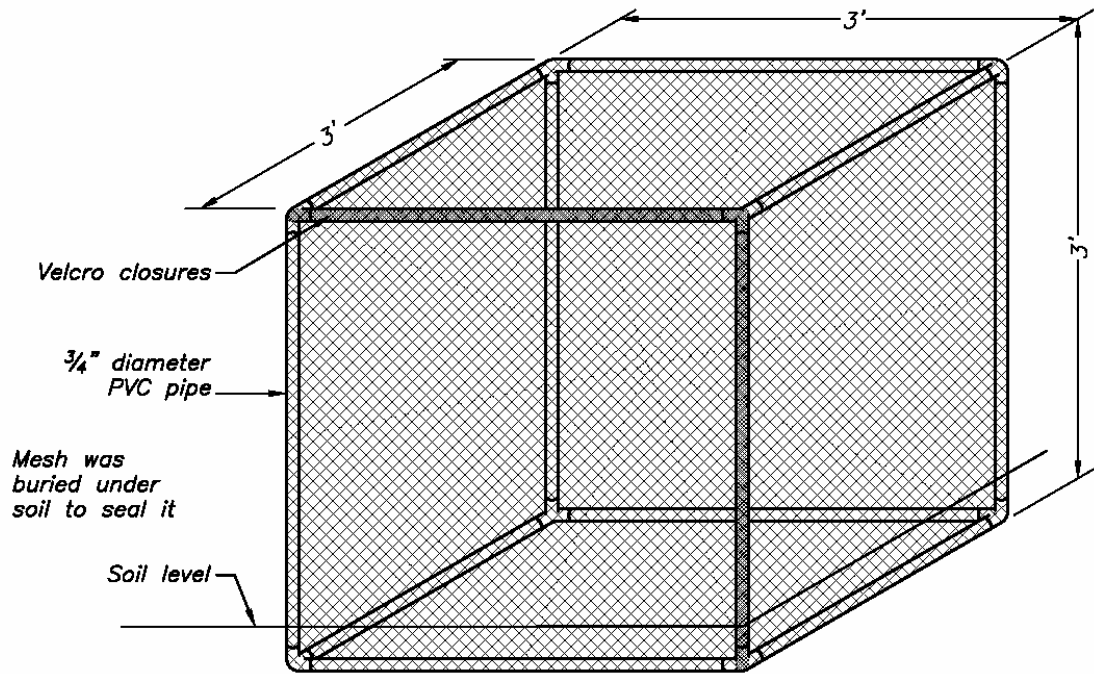


Fig. 2. Design of exclusion cages in 2004. The cage frame alone was used as a control for cage effects. Figure by Kelly Schweikert.

Aphid Infestation. Each exclusion cage was infested with 15 apterous viviparous soybean aphid nymphs that had been deposited within 48 h of infestation using the following procedure: alate soybean aphids were placed on excised soybean leaves in petri dishes with lids with moist filter paper for 48 hours. After this period, the alates were removed and the remaining nymphs were transferred using a camel's hair brush to infest the exclusion cages. This was done to assure even age of nymphs and also to mimic an alate's behavior of depositing nymphs then moving to another plant, as suggested by Liu et al. (2004). Cages were infested at three different times: 21 July, 5 August and 3 September. Control cages were uninfested. Infestation times were selected to simulate the typical arrival times of migrant soybean aphids.

Data were collected in approximate seven day intervals from 28 July until 29 September. On each sample date, temperature and relative humidity inside each cage were measured at canopy height by inserting a probe (EasyView 20; Extech Instruments Corp., Waltham, MA) through the Velcro® before opening the cage. Number of thrips per leaf were estimated on a scale of zero to four; 0=0 thrips per leaf, 1=1-10 thrips per leaf, 2=11-25 thrips per leaf, 3=26-75 thrips per leaf and 4=>75 thrips per leaf. Soybean aphid populations early in the season were directly counted. Once population became large, soybean aphid numbers were estimated by sampling several leaves, averaging the number of aphids, then multiplying by the number of leaves on the plant. Predatory insects were directly counted; predators which should not be present were then removed. Additionally, the height of each plant in the cage was measured and plant phenology was recorded using the method by Fehr et al. (1971).

Statistical Analysis. Temperature, relative humidity, and plant height were analyzed using repeated measures analysis of variance. Soybean aphid and predator counts were square root transformed and then analyzed using SAS mixed procedure (SAS 2001) (Appendix 1). The basic analysis of variance was a randomized complete block design. The linear statistical model contains the effect of small mesh, medium mesh, large mesh, and no mesh. Each infestation (July 21, August 5, September 3, and uninfested cages) was analyzed separately. The sub-plot contained the effect of date and all possible interactions with the main plot effects. Differences between means were determined using Fisher's least significant difference (LSD). Analysis of variance tables contain results from transformed data while graphs are of untransformed data. The rate of increase of soybean aphid populations in cages of different mesh sizes was analyzed using a program supplied by Missouri Agricultural Experiment Station statisticians. Program equations are as outlined by Snedecor and Cochran (1989). Slope from infestation to peak of population growth was determined and compared.

Results

***Aphis glycines* Population Growth.** Slopes and R^2 values are reported in Table 1. A summary of soybean aphid populations by infestation date are reported in figure 3.

2004. **Table 1. Slope and R² values for *A. glycines* populations until peak during exclusion trials,**

Treatment	Slope	R ²	
July 21 Infestation			
Small	15808.0	0.7447	a
Medium	3151.1	0.5345	b
Large	984.1	0.4368	b
No	0.4	0.7321	c
August 5 Infestation			
Small	151.5	0.5753	a
Medium	233.2	0.4991	a
Large	3.9	0.5026	b
No	1.2	0.2084	b
September 3 Infestation			
Small	0.2	0.1182	a
Medium	-1.7	0.4854	a
Large	-0.7	0.1099	a
No	0.0	0.0117	a
Uninfested			
Small	0.0	0.3693	b
Medium	0.2	0.3491	ab
Large	0.3	0.3453	ab
No	1.0	0.3946	a

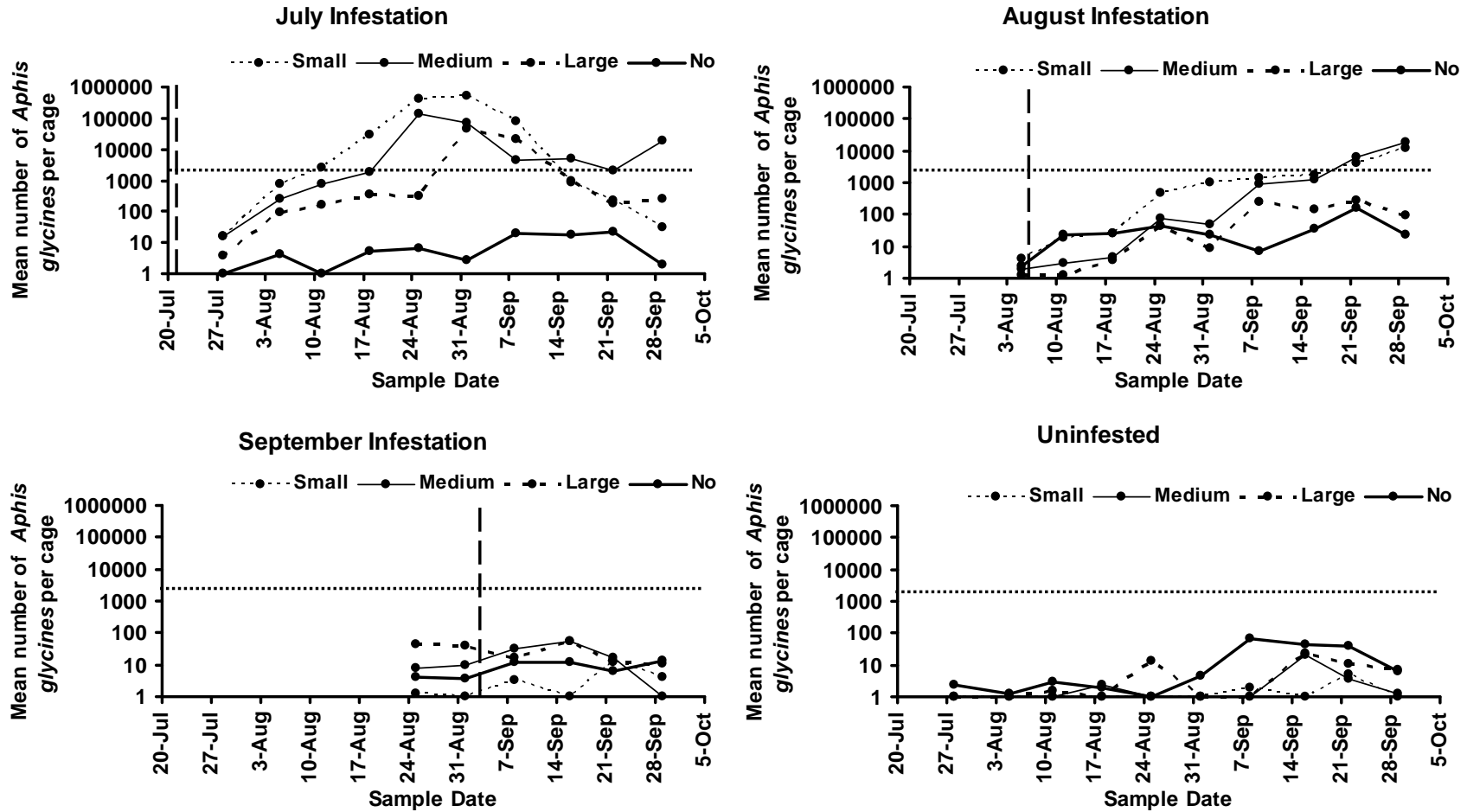


Fig. 3. Summary of *A. glycines* populations by infestation date. Vertical dashed line indicates infestation date. Horizontal dotted line indicates threshold of 250 aphids per plant.

Predator Abundance. Predators occurring in exclusion cages that may contribute to soybean aphid population suppression and their percent abundance are reported in Table 2. Predator abundance by infestation and sample date are outlined in tables 3-6. Category 'Other' includes tiger beetles (Coleoptera: Carabidae), ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), big eyed bugs (Hemiptera: Geocoridae), and aphid midges (Diptera: Cecidomyiidae); all of which were found in very small numbers.

Table 2. Potential *A. glycines* predators and their percent abundance during exclusion trials, 2004.

Order		%
Family	Insect	Abundance
Heteroptera		
Anthocoridae	<i>Orius insidiosus</i> (Say) Adults	17.5
	<i>Orius insidiosus</i> Nymphs	22.0
Total percent		39.5
Coleoptera		
Coccinellidae	Ladybird Adult	12.5
	Ladybird Larvae	12.8
	Ladybird Pupae	8.7
	Ladybird Eggs	3.4
Total percent		37.4
Diptera		
Syrphidae	Syrphid spp. Adult	4.5
	Syrphid spp. Larvae	2.7
	Syrphid spp. Egg	2.3
Total percent		9.6
Neuroptera		
Chrysopidae	<i>Chrysoperla</i> spp. Adults	1.3
	<i>Chrysoperla</i> spp. Larvae	1.0
	<i>Chrysoperla</i> spp. Egg	1.9
Total percent		4.2
Other		9.2
Total percent		9.2

Table 3. Potential *A. glycines* predators and their percent abundance by sample date among July 21 infested cages.

Mesh	Insect	Sample Date																			
		28-Jul		5-Aug		11-Aug		18-Aug		25-Aug		1-Sep		8-Sep		16-Sep		22-Sep		29-Sep	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Small	<i>Orius insidiosus</i>	0	0.0	3	100.0	0	0.0	1	100.0	0	0.0	2	100.0	0	0.0	2	100.0	1	11.1	0	0.0
	Coccinellid	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	7	77.8	1	25.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	11.1	1	25.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	5	100.0	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	2	100.0	0	0.0	0	0.0	2	50.0
Medium	<i>Orius insidiosus</i>	21	100.0	8	100.0	12	100.0	11	91.7	3	27.3	6	54.5	11	55.0	2	6.1	1	5.0	0	0.0
	Coccinellid	0	0.0	0	0.0	0	0.0	1	8.3	6	54.5	4	36.4	5	25.0	16	48.5	13	65.0	0	0.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	10.0	13	39.4	5	25.0	0	0.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	1	9.1	0	0.0	2	10.0	0	0.0	1	5.0	0	0.0
	Other	0	0.0	0	0.0	0	0.0	0	0.0	1	9.1	1	9.1	0	0.0	2	6.1	0	0.0	0	0.0
Large	<i>Orius insidiosus</i>	22	100.0	6	75.0	17	94.4	20	74.1	7	70.0	1	33.3	4	11.4	1	2.8	2	28.6	0	0.0
	Coccinellid	0	0.0	0	0.0	0	0.0	7	25.9	3	30.0	0	0.0	31	88.6	27	75.0	5	71.4	7	100.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	13.9	0	0.0	0	0.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	33.3	0	0.0	0	0.0	0	0.0	0	0.0
	Other	0	0.0	2	25.0	1	5.6	0	0.0	0	0.0	1	33.3	0	0.0	3	8.3	0	0.0	0	0.0
Control	<i>Orius insidiosus</i>	15	100.0	3	100.0	1	20.0	4	66.7	2	66.7	0	0.0	1	100.0	2	66.7	4	57.1	0	0.0
	Coccinellid	0	0.0	0	0.0	0	0.0	1	16.7	1	33.3	0	0.0	0	0.0	1	33.3	2	28.6	1	100.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	14.3	0	0.0
	Other	0	0.0	0	0.0	4	80.0	1	16.7	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 4. Potential *A. glycines* predators and their percent abundance by sample date among August 5 infested cages.

Mesh	Insect	Sample Date																			
		28-Jul		5-Aug		11-Aug		18-Aug		25-Aug		1-Sep		8-Sep		16-Sep		22-Sep		29-Sep	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Small	<i>Orius insidiosus</i>	-	-	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	6	14.0
	Coccinellid	-	-	5	83.3	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	35	81.4
	Syrphidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	1	16.7	0	0.0	0	0.0	0	0.0	1	100.0	2	100.0	0	0.0	0	0.0	2	4.7
Medium	<i>Orius insidiosus</i>	-	-	10	76.9	9	90.0	8	88.9	3	20.0	0	0.0	1	33.3	0	0.0	0	0.0	1	100.0
	Coccinellid	-	-	2	15.4	0	0.0	1	11.1	6	40.0	1	33.3	1	33.3	0	0.0	2	66.7	0	0.0
	Syrphidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	1	33.3	0	0.0	0	0.0	1	33.3	0	0.0
	Other	-	-	1	7.7	1	10.0	0	0.0	6	40.0	1	33.3	1	33.3	2	100.0	0	0.0	0	0.0
Large	<i>Orius insidiosus</i>	-	-	6	85.7	6	85.7	15	93.8	3	42.9	2	33.3	0	0.0	3	100.0	3	50.0	0	0.0
	Coccinellid	-	-	0	0.0	0	0.0	0	0.0	2	28.6	0	0.0	1	50.0	0	0.0	1	16.7	2	100.0
	Syrphidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	0	0.0	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	1	14.3	1	14.3	1	6.3	1	14.3	4	66.7	1	50.0	0	0.0	2	33.3	0	0.0
Control	<i>Orius insidiosus</i>	-	-	1	100.0	6	75.0	14	87.5	4	50.0	3	75.0	1	100.0	4	66.7	1	50.0	0	0.0
	Coccinellid	-	-	0	0.0	2	25.0	2	12.5	3	37.5	1	25.0	0	0.0	1	16.7	1	50.0	1	100.0
	Syrphidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	0	0.0	0	0.0	0	0.0	1	12.5	0	0.0	0	0.0	1	16.7	0	0.0	0	0.0

Table 5. Potential *A. glycines* predators and their percent abundance by sample date among September 3 infested cages.

Mesh	Insect	Sample Date																			
		28-Jul		5-Aug		11-Aug		18-Aug		25-Aug		1-Sep		8-Sep		16-Sep		22-Sep		29-Sep	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Small	<i>Orius insidiosus</i>	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	20	33.3
	Coccinellid	-	-	-	-	-	-	-	-	3	60.0	4	100.0	0	0.0	0	0.0	0	0.0	39	65.0
	Syrphidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	-	-	-	-	-	-	2	40.0	0	0.0	0	0.0	0	0.0	1	100.0	1	1.7
Medium	<i>Orius insidiosus</i>	-	-	-	-	-	-	-	-	2	66.7	3	75.0	4	100.0	2	28.6	1	100.0	0	0.0
	Coccinellid	-	-	-	-	-	-	-	-	1	33.3	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0
	Syrphidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	-	-	-	-	-	-	0	0.0	1	25.0	0	0.0	4	57.1	0	0.0	0	0.0
Large	<i>Orius insidiosus</i>	-	-	-	-	-	-	-	-	2	66.7	1	25.0	2	66.7	13	76.5	0	0.0	0	0.0
	Coccinellid	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	4	23.5	0	0.0	1	100.0
	Syrphidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	-	-	-	-	-	-	1	33.3	3	75.0	1	33.3	0	0.0	1	100.0	0	0.0
Control	<i>Orius insidiosus</i>	-	-	-	-	-	-	-	-	4	80.0	1	50.0	1	33.3	5	100.0	0	0.0	0	0.0
	Coccinellid	-	-	-	-	-	-	-	-	1	20.0	0	0.0	1	33.3	0	0.0	0	0.0	0	0.0
	Syrphidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	-	-	-	-	-	-	-	-	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	-	-	-	-	-	-	-	-	0	0.0	1	50.0	1	33.3	0	0.0	0	0.0	0	0.0

Table 6. Potential *A. glycines* predators and their percent abundance by sample date among uninfested cages.

Mesh	Insect	Sample Date																			
		28-Jul		5-Aug		11-Aug		18-Aug		25-Aug		1-Sep		8-Sep		16-Sep		22-Sep		29-Sep	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Small	<i>Orius insidiosus</i>	1	100.0	2	50.0	0	0.0	0	0.0	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	1	2.1
	Coccinellid	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	1	50.0	1	25.0	1	100.0	1	100.0	32	66.7
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	12.5
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	0	0.0	2	50.0	3	100.0	0	0.0	1	100.0	0	0.0	3	75.0	0	0.0	0	0.0	9	18.8
Medium	<i>Orius insidiosus</i>	19	100.0	3	75.0	7	70.0	13	92.9	1	50.0	0	0.0	3	75.0	3	100.0	0	0.0	0	0.0
	Coccinellid	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	25.0	0	0.0	1	100.0	0	0.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	0	0.0	1	25.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	0	0.0	0	0.0	3	30.0	1	7.1	1	50.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0
Large	<i>Orius insidiosus</i>	18	100.0	3	75.0	8	72.7	19	100.0	3	37.5	2	25.0	0	0.0	1	9.1	2	100.0	0	0.0
	Coccinellid	0	0.0	0	0.0	1	9.1	0	0.0	3	37.5	0	0.0	1	50.0	8	72.7	0	0.0	1	100.0
	Syrphidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	2	25.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Other	0	0.0	1	25.0	2	18.2	0	0.0	0	0.0	6	75.0	1	50.0	2	18.2	0	0.0	0	0.0
Control	<i>Orius insidiosus</i>	7	100.0	1	50.0	1	50.0	6	100.0	0	0.0	1	50.0	2	28.6	4	80.0	3	60.0	0	0.0
	Coccinellid	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	28.6	0	0.0	1	20.0	1	100.0
	Syrphidae	0	0.0	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chrysopidae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0	0	0.0
	Other	0	0.0	1	50.0	0	0.0	0	0.0	1	100.0	1	50.0	2	28.6	1	20.0	1	20.0	0	0.0

July 21 Infestation.

Aphids. Soybean aphid populations differed significantly across mesh, sample date, and mesh \times sample date (Table 7). Populations did not differ significantly across replication. Populations in cages with small small mesh increased to a peak on 1 September and then declined until the end of sampling on 29 September (Fig. 4). Populations in cages with medium and large mesh increased for a time, peaking on 25 August and 1 September, respectively, then declined until the end of sampling on 29 September. Prior to infestation on 21 July, no soybean aphids were observed in any cage. After infestation, cages with no mesh maintained very low population of soybean aphids while other cages had significant increases in soybean aphid populations (Fig. 4).

Predators. *Orius insidiosus* populations differed significantly with sample date and mesh \times sample date (Table 7). Populations did not differ significantly with replication or mesh. Populations were highest early in the season and declined until the end of sampling (Fig 5). Coccinellid populations differed significantly with sample date but did not differ significantly with replication, mesh, or mesh \times sample date (Table 7). Coccinellid populations remained low until late August when they rose and then declined until the end of sampling (Fig. 6).

Temperature, Relative Humidity, and Soybean Growth. Temperature differed significantly across replication and mesh (Table 7). Relative humidity differed significantly by replication (Table 7). Plant height differed significantly by mesh and mesh \times sample date (Table 7). Temperature, relative humidity, and plant height differed significantly by sample date. Temperature and relative humidity differed significantly between mesh on few sampling dates (Fig. 7 and Fig. 8). There was a significant

difference in plant height between no mesh and all other treatments on all sampling dates (Fig. 9). Cages with small, medium, and large mesh also differed significantly in plant height on several sampling dates.

Table 7. Sources of variation, degrees of freedom (numerator, denominator), *F* statistics, and probabilities for the effects of mesh on temperature, relative humidity, plant height, *A. glycines*, *O. insidiosus*, and Coccinellid numbers in July 21 infested cages during *A. glycines* exclusion trials, 2004.

Effect	df	<i>F</i>	<i>P</i>
Temperature			
Replication	3,9	6.29	0.0137
Mesh	3,9	19.8	0.0003
Sample Date	9,107	300.5	<0.0001
Mesh × Sample Date	27,107	1.0	0.4671
Relative Humidity			
Replication	3,9	4.8	0.0296
Mesh	3,9	2.9	0.0961
Sample Date	9,107	168.1	<0.0001
Mesh × Sample Date	27,107	1.5	0.0896
Plant Height			
Replication	3,9	3.6	0.0600
Mesh	3,9	119.8	<0.0001
Sample Date	9,101	185.8	<0.0001
Mesh × Sample Date	27,101	11.4	<0.0001
<i>A. glycines</i>			
Replication	3,9	0.6	0.6534
Mesh	3,9	5.4	0.0214
Sample Date	9,107	7.8	<0.0001
Mesh × Sample Date	27,107	2.5	0.0005
<i>O. insidiosus</i>			
Replication	3,9	0.1	0.9548
Mesh	3,9	3.4	0.0667
Sample Date	9,107	4.7	<0.0001
Mesh × Sample Date	27,107	2.6	0.0003
Coccinellid			
Replication	3,9	1.4	0.2961
Mesh	3,9	0.7	0.5866
Sample Date	9,107	4.1	0.0002
Mesh × Sample Date	27,107	0.9	0.6059

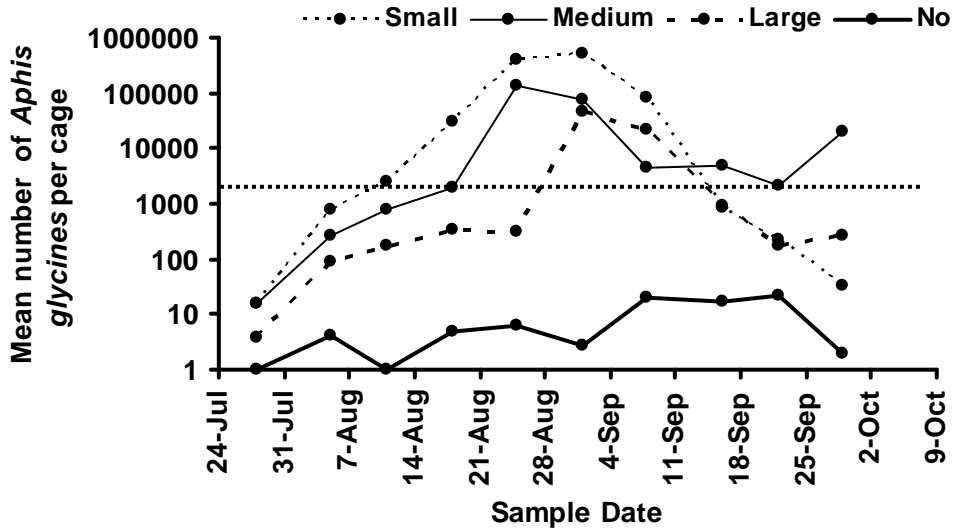


Fig. 4. Mean number of *A. glycines* per cage in small, medium, large, and control cage treatments in July 21 infested cages. Horizontal dotted line indicates threshold of 250 aphids per plant.

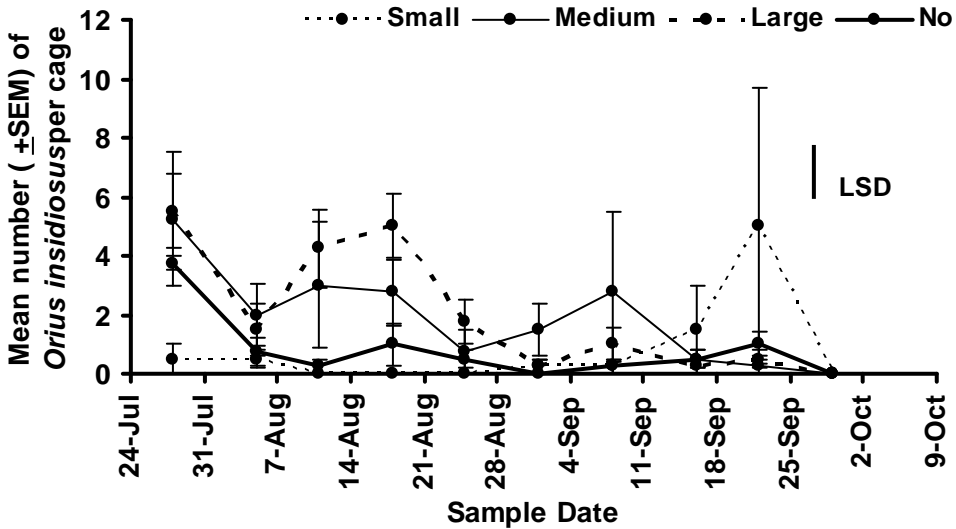


Fig. 5. Mean number of *O. insidiosus* per cage in small, medium, large, and control cage treatments in July 21 infested cages. LSD=1.8 ($P=0.05$).

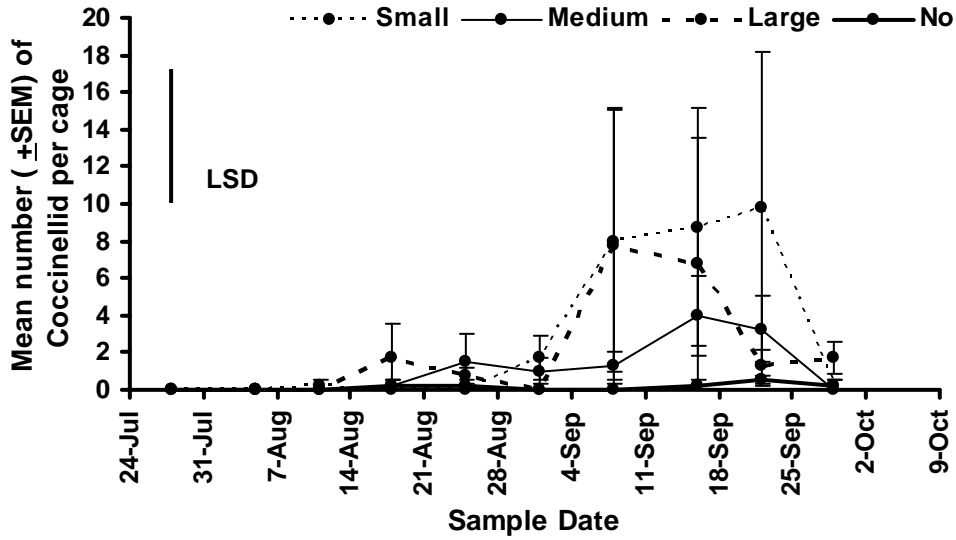


Fig. 6. Mean number of Coccinellid per cage in small, medium, large, and control cage treatments in July 21 infested cages. LSD=7.5 ($P=0.05$).

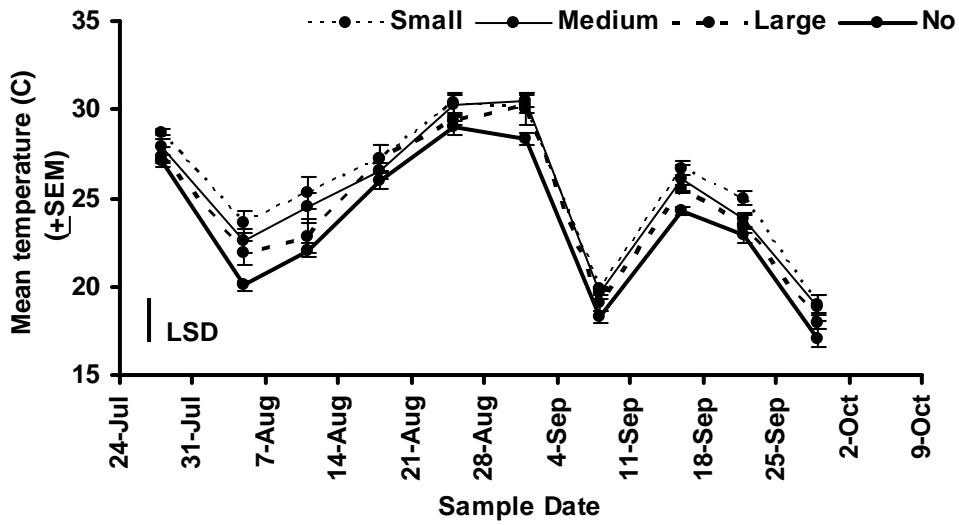


Fig. 7. Mean temperature ($^{\circ}\text{C}$) in small, medium, large, and control cage treatments in July 21 infested cages. LSD=2.5 ($P=0.05$).

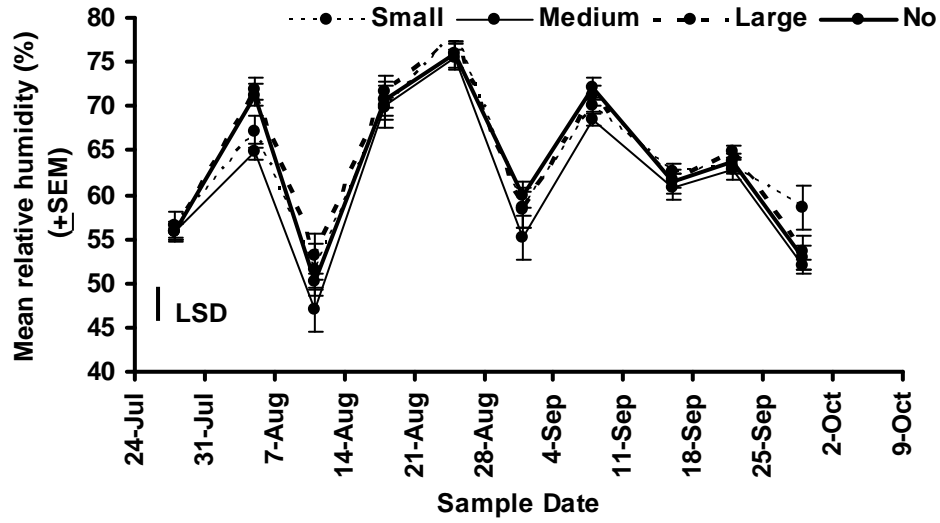


Fig. 8. Mean relative humidity (%) in small, medium, large, and control cage treatments in July 21 infested cages. LSD=4.1 ($P=0.05$).

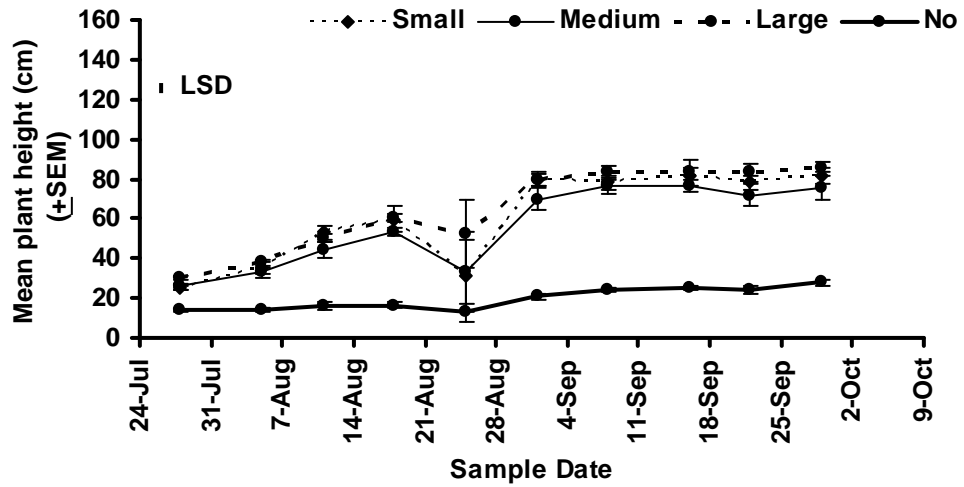


Fig. 9. Mean plant height (cm) in small, medium, large, and control cage treatments in July 21 infested cages. LSD=3.2 ($P=0.05$).

August 5 Infestation.

Aphids. Soybean aphid populations differed significantly across mesh, sample date, and mesh \times sample date (Table 8). Populations in cages with small and medium mesh increased to a peak on the last sample date, 29 September. In cages with large mesh and cages with no mesh, populations increased to a peak on 22 September, then decreased until the end of sampling on 29 September. Prior to infestation, no soybean aphids were observed in any exclusion cages. After infestations, populations in all cages began rising (Fig. 10). Cages with small and medium mesh eventually reached the economic threshold of 250 aphids per plant.

Predators. *Orius insidiosus* populations differed significantly across sample date but did not differ significantly across replication, mesh, or mesh \times sample date (Table 8). Populations peaked early in the season then decreased until the end of sampling (Fig.11). Coccinellid populations did not differ significantly across replication, mesh, sample date, or mesh \times sample date (Table 8). Populations remained low throughout the sampling in all cages (Fig. 12).

Temperature, Relative Humidity, and Soybean Growth. Temperature differed significantly across replication and mesh (Table 8). Temperature and relative humidity differed significantly between treatments on few sample date (Fig. 13 and Fig. 14). Relative humidity differed significantly by replication and mesh (Table 8). Plant height differed significantly by mesh (Table 8). There was a significant difference in plant height between cages with no mesh and all mesh size cages on all sampling dates (Fig. 14). Cages with small, medium, and large mesh also differed significantly in plant height

on one sampling dates. Temperature, relative humidity, and plant height differed significantly by sample date.

Table 8. Sources of variation, degrees of freedom (numerator, denominator), *F* statistics, and probabilities for the effects of mesh on temperature, relative humidity, plant height, *A. glycines*, *O. insidiosus*, and Coccinellid numbers in August 5 infested cages during *A. glycines* exclusion trials, 2004.

Effect	df	<i>F</i>	<i>P</i>
Temperature			
Replication	3,9	6.39	0.0131
Mesh	3,9	8.4	0.0057
Sample Date	7,84	307.4	<0.0001
Mesh × Sample Date	21,84	1.1	0.3441
Relative Humidity			
Replication	3,9	9.8	0.0034
Mesh	3,9	4.2	0.0409
Sample Date	7,84	195.4	<0.0001
Mesh × Sample Date	21,84	1.6	0.0833
Plant Height			
Replication	3,9	0.9	0.4972
Mesh	3,9	7.3	0.0088
Sample Date	7,80	15.4	<0.0001
Mesh × Sample Date	21,80	1.4	0.1494
<i>A. glycines</i>			
Replication	3,9	2.7	0.1084
Mesh	3,9	4.5	0.0340
Sample Date	7,84	7.6	<0.0001
Mesh × Sample Date	21,84	2.2	0.0061
<i>O. insidiosus</i>			
Replication	3,9	0.4	0.7277
Mesh	3,9	2.4	0.1379
Sample Date	7,84	5.4	<0.0001
Mesh × Sample Date	21,84	1.3	0.2137
Coccinellid			
Replication	3,9	1.3	0.3270
Mesh	3,9	0.2	0.9169
Sample Date	7,84	2.0	0.0688
Mesh × Sample Date	21,84	0.5	0.9682

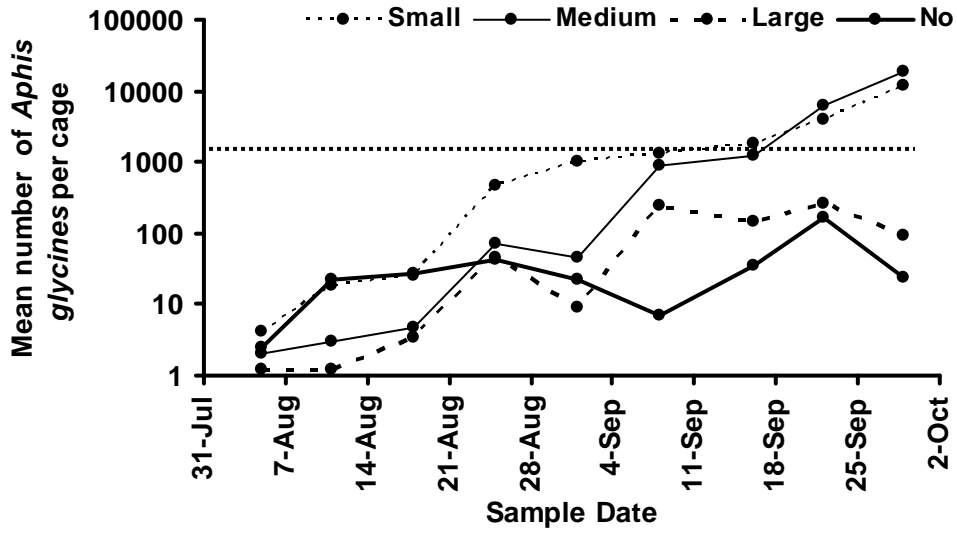


Fig. 10. Mean number of *A. glycines* per cage in small, medium, large, and control cage treatments in August 5 infested cages. Horizontal dotted line indicates threshold of 250 aphids per plant.

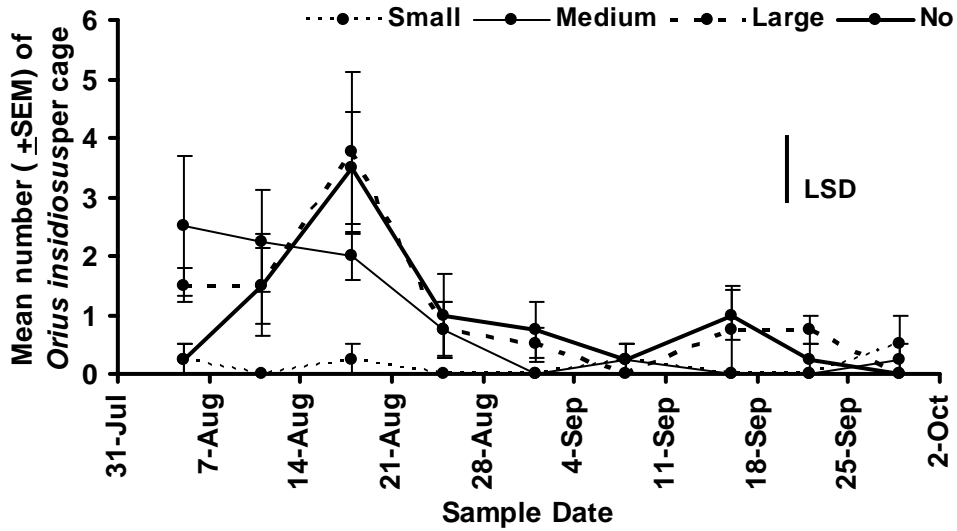


Fig. 11. Mean number of *O. insidiosus* per cage in small, medium, large, and control cage treatments in August 5 infested cages. LSD=1.2 ($P=0.05$).

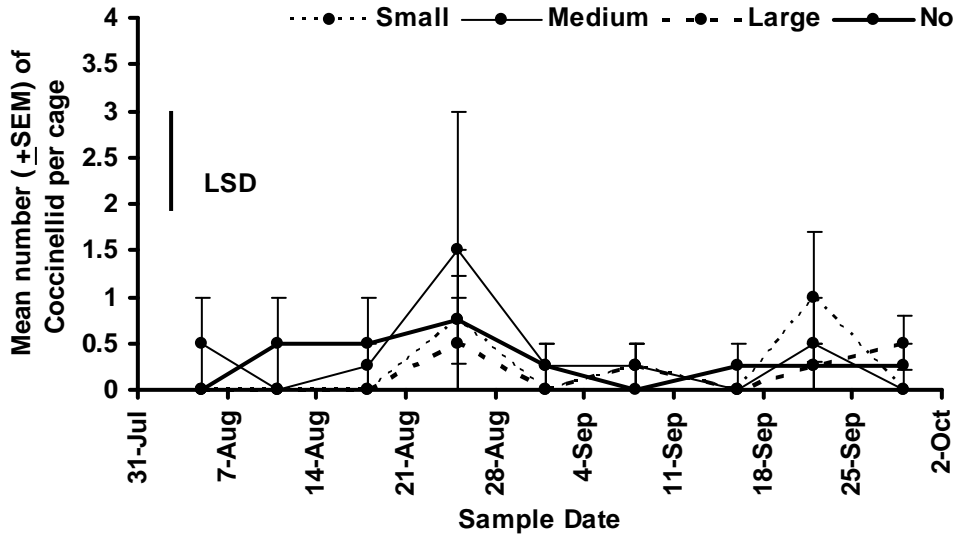


Fig. 12. Mean number of Coccinellid per cage in small, medium, large, and control cage treatments in August 5 infested cages. LSD=1.1 ($P=0.05$).

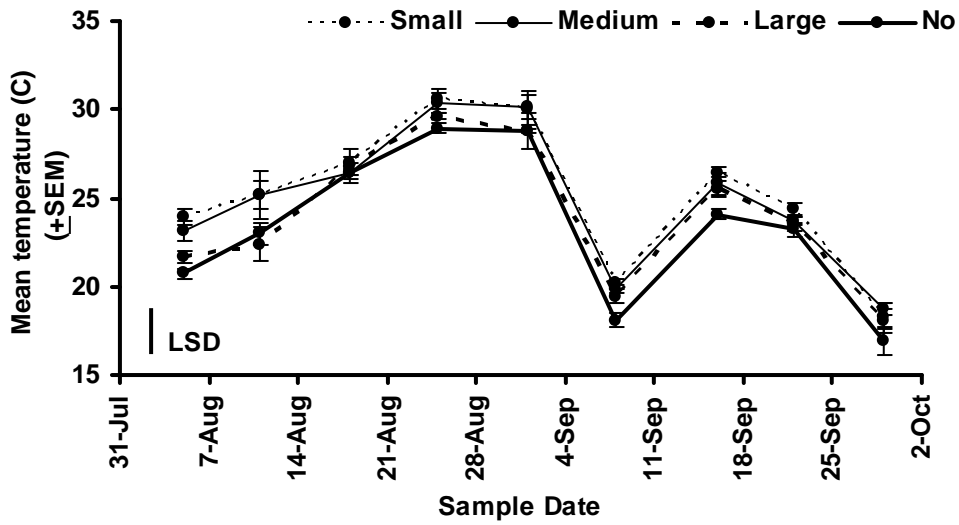


Fig. 13. Mean temperature (°C) in small, medium, large, and control cage treatments in August 5 infested cages. LSD=2.6 ($P=0.05$).

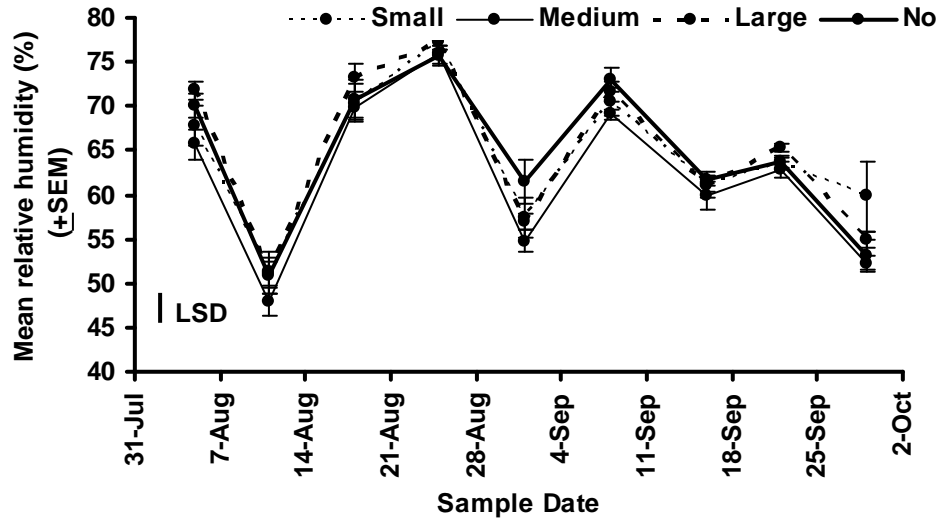


Fig. 14. Mean relative humidity (%) in small, medium, large, and control cage treatments in August 5 infested cages. LSD=3.7 ($P=0.05$).

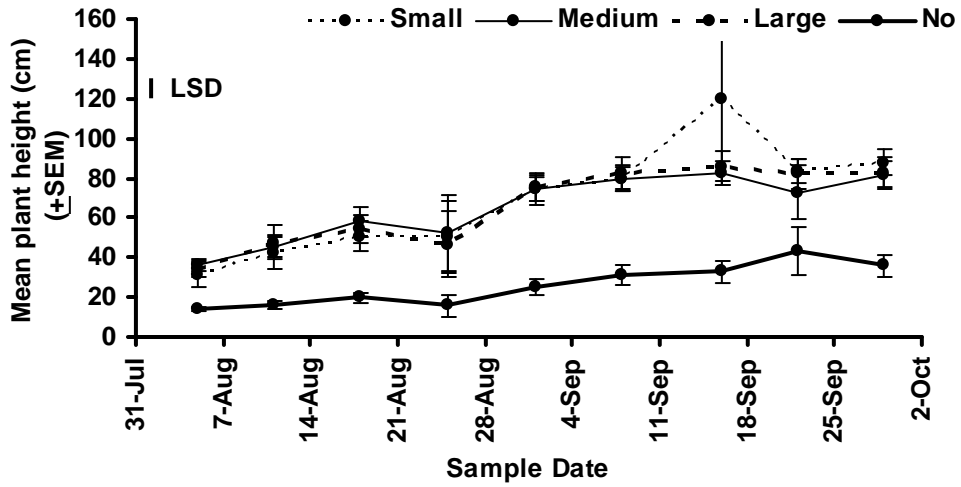


Fig. 15. Mean plant height (cm) in small, medium, large, and control cage treatments in August 5 infested cages. LSD=11.8 ($P=0.05$).

September 3 Infestation.

Aphids. Soybean aphid populations did not differ significantly across any of the effects (Table 9). None of the treatments had high populations of soybean aphid and none reached threshold (Fig. 16).

Predators. *Orius insidiosus* populations did not differ significantly across any of the effects (Table 9). Coccinellid populations did not differ significantly across replication, mesh, sample date, or mesh \times sample date (Table 9). *Orius insidiosus* (Fig. 17) and coccinellid (Fig. 18) populations were relatively stable through the season, peaking on 16 September.

Temperature, Relative Humidity, and Soybean Growth. Temperature differed significantly across mesh (Table 9). Relative humidity did not differ except by sample date (Table 9). Temperature (Fig. 19) and relative humidity (Fig. 20) were consistent throughout sampling, differing significantly on only one sampling date. Plant height differed significantly by mesh (Table 9). Plant height in cages with no mesh was significantly different from all other treatments throughout the sampling period (Fig. 21). Temperature, relative humidity, and plant height differed significantly by sample date.

Table 9. Sources of variation, degrees of freedom (numerator, denominator), *F* statistics, and probabilities for the effects of mesh on temperature, relative humidity, plant height, *A. glycines*, *O. insidiosus*, and Coccinellid numbers in September 3 infested cages during *A. glycines* exclusion trials, 2004.

Effect	df	<i>F</i>	<i>P</i>
Temperature			
Replication	3,9	3.23	0.0748
Mesh	3,9	10.2	0.0030
Sample Date	3,36	253.0	<0.0001
Mesh × Sample Date	9,36	1.2	0.3232
Relative Humidity			
Replication	3,9	3.4	0.0656
Mesh	3,9	3.3	0.0705
Sample Date	3,36	66.1	<0.0001
Mesh × Sample Date	9,36	1.0	0.4815
Plant Height			
Replication	3,9	2.0	0.1816
Mesh	3,9	45.2	<0.0001
Sample Date	3,36	3.5	0.0245
Mesh × Sample Date	9,36	0.7	0.7378
<i>A. glycines</i>			
Replication	3,9	0.1	0.9389
Mesh	3,9	0.3	0.8349
Sample Date	3,36	1.5	0.2318
Mesh × Sample Date	9,36	0.9	0.5447
<i>O. insidiosus</i>			
Replication	3,9	0.5	0.6697
Mesh	3,9	0.8	0.5199
Sample Date	3,36	1.7	0.1797
Mesh × Sample Date	9,36	1.3	0.2820
Coccinellid			
Replication	3,9	0.5	0.6649
Mesh	3,9	0.4	0.7445
Sample Date	3,36	0.8	0.4936
Mesh × Sample Date	9,36	1.1	0.4139

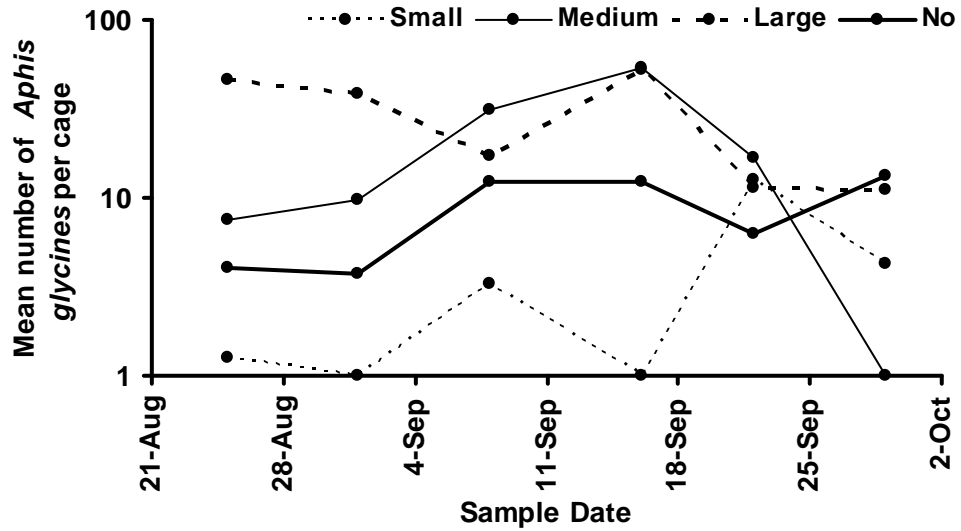


Fig. 16. Mean number of *A. glycines* per cage in small, medium, large, and control cage treatments in September 3 infested cages.

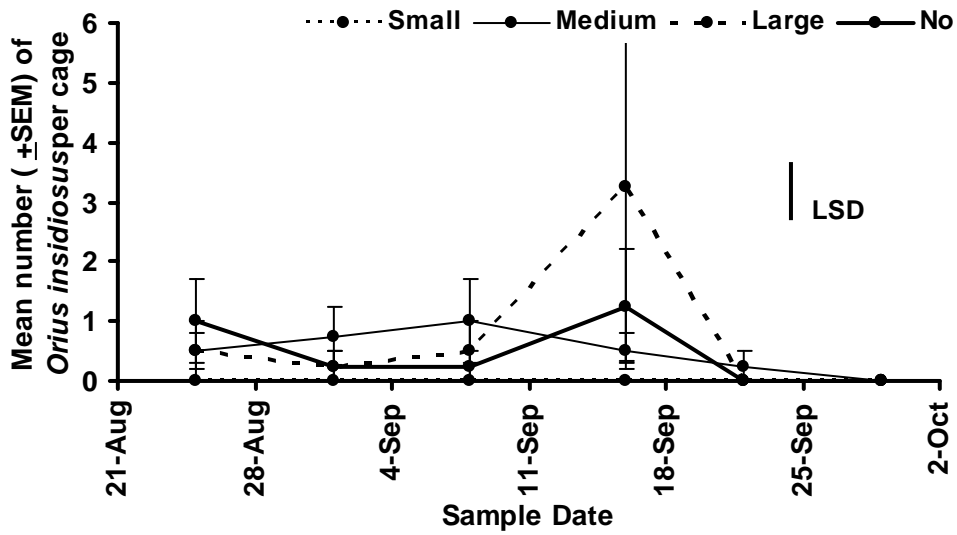


Fig. 17. Mean number of *O. insidiosus* per cage in small, medium, large, and control cage treatments in September 3 infested cages. LSD=1.0 ($P=0.05$).

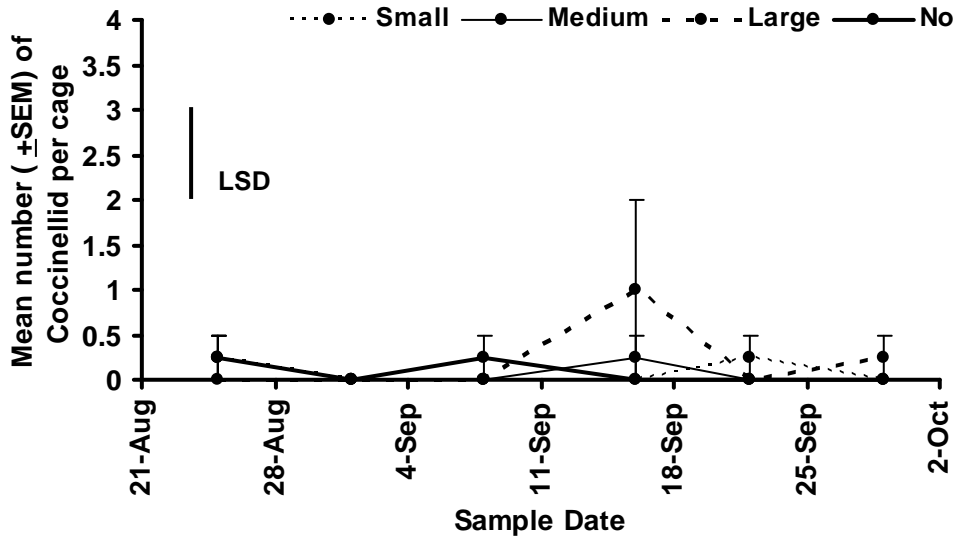


Fig. 18. Mean number of Coccinellid per cage in small, medium, large, and control cage treatments in September 3 infested cages. LSD=0.8 ($P=0.05$).

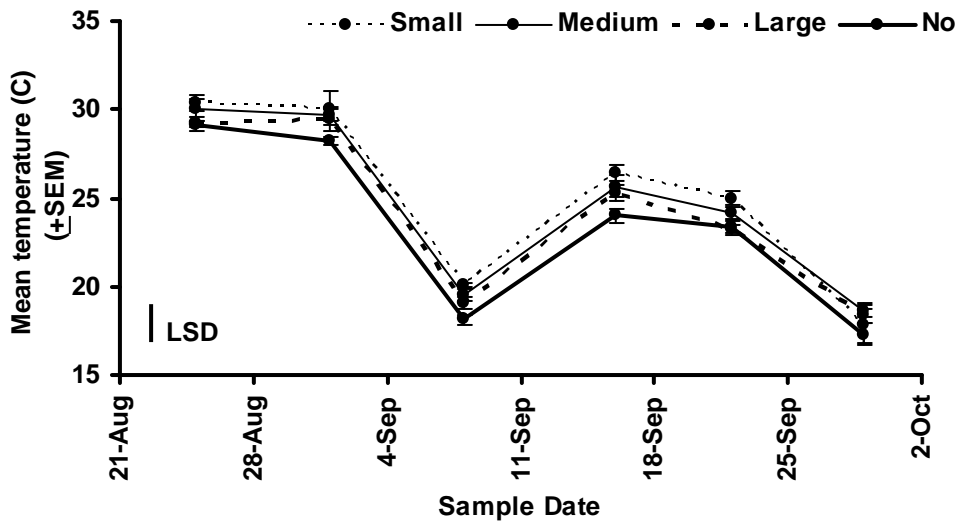


Fig. 19. Mean temperature (°C) in small, medium, large, and control cage treatments in September 3 infested cages. LSD=2.3 ($P=0.05$).

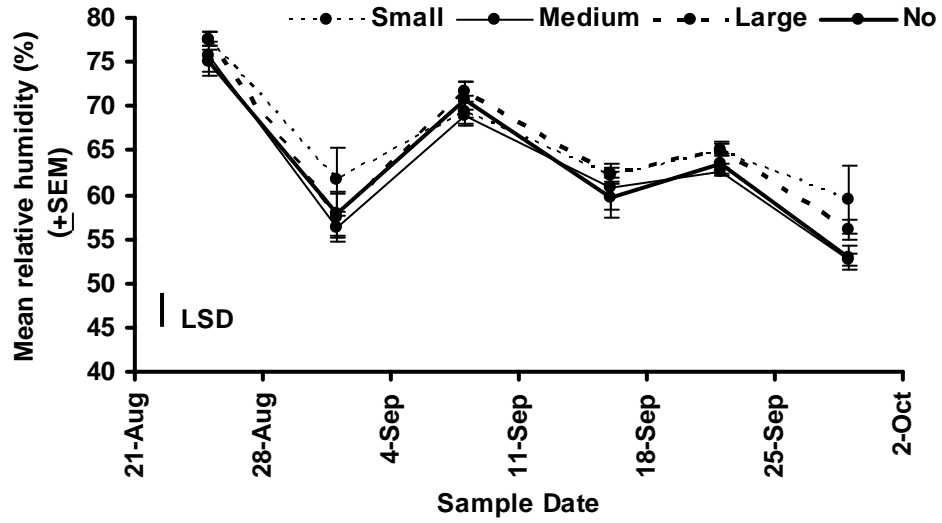


Fig. 20. Mean relative humidity (%) in small, medium, large, and control cage treatments in September 3 infested cages. LSD=4.3 ($P=0.05$).

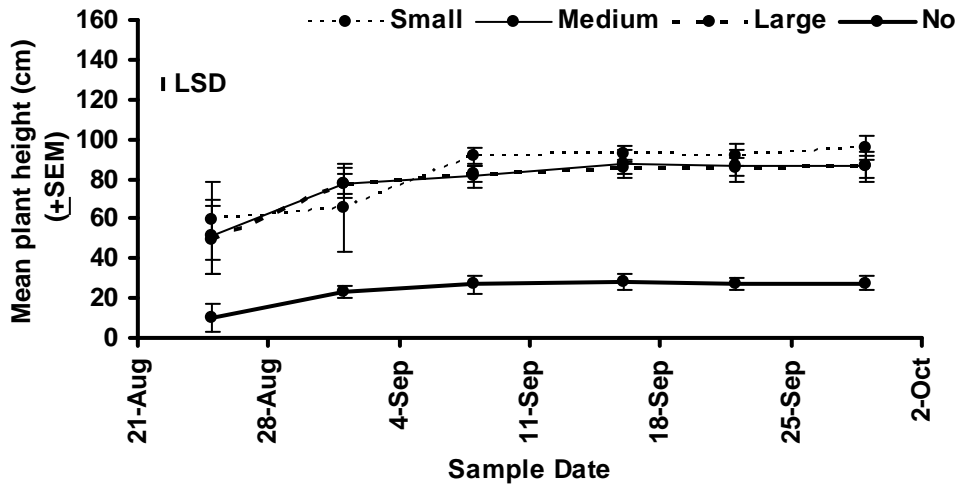


Fig. 21. Mean plant height (cm) in small, medium, large, and control cage treatments in September 3 infested cages. LSD=5.3 ($P=0.05$).

Uninfested Cages.

Aphids. Soybean aphid populations differed significantly across mesh and sample date (Table 10). Cages with small and medium mesh, though designed to exclude aphids, contained a very small number of aphids, less than 15 (Fig. 22). This may be the result of aphids being transferred on the observer from a heavily infested cage to another cage. Exclusion cages with medium mesh had low numbers of aphids throughout the sampling period. Cages with no mesh had the highest number of aphids on 8 September then declined until the last sample date.

Predators. *Orius insidiosus* populations differed significantly across mesh and sample date (Table 10). Populations were highest early in the season where they peaked twice then declined the rest of the sampling period (Fig. 23). Coccinellid populations differed significantly across mesh \times sample date (Table 10). Populations did not differ across replication, mesh, or sample date (Table 10). Cages maintained very low numbers of coccinellids throughout the season (Fig. 24).

Temperature, Relative Humidity, and Soybean Growth. Temperature differed significantly across mesh and mesh \times sample date (Table 10). Temperature differed significantly between treatments on one sample date (Fig. 25). Relative humidity differed significantly by replication (Table 10). Relative humidity differed significantly between treatments on three sample dates (Fig. 26). Plant height differed significantly by mesh and mesh \times sample date (Table 10). Temperature, relative humidity, and plant height differed significantly by sample date. Plant height in cages with no mesh differed significantly from small, medium, and large mesh cages on all sample dates (Fig. 27).

Table 10. Sources of variation, degrees of freedom (numerator, denominator), *F* statistics, and probabilities for the effects of mesh on temperature, relative humidity, plant height, *A. glycines*, *O. insidiosus*, and Coccinellid numbers in uninfested cages during *A. glycines* exclusion trials, 2004.

Effect	df	<i>F</i>	<i>P</i>
Temperature			
Replication	3,9	3.71	0.0551
Mesh	3,9	9.9	0.0033
Sample Date	9,108	288.1	<0.0001
Mesh × Sample Date	27,108	1.6	0.0480
Relative Humidity			
Replication	3,9	4.9	0.0278
Mesh	3,9	3.6	0.0580
Sample Date	9,108	139.0	<0.0001
Mesh × Sample Date	27,108	1.2	1.2400
Plant Height			
Replication	3,9	2.2	0.1586
Mesh	3,9	23.9	0.0001
Sample Date	9,102	39.5	<0.0001
Mesh × Sample Date	27,102	3.1	<0.0001
<i>A. glycines</i>			
Replication	3,9	3.3	0.0734
Mesh	3,9	6.8	0.0110
Sample Date	9,108	5.1	<0.0001
Mesh × Sample Date	27,108	1.5	0.0649
<i>O. insidiosus</i>			
Replication	3,9	0.9	0.4663
Mesh	3,9	4.1	0.0423
Sample Date	9,108	4.3	<0.0001
Mesh × Sample Date	27,108	1.1	0.4081
Coccinellid			
Replication	3,9	0.3	0.8434
Mesh	3,9	1.6	0.2614
Sample Date	9,108	1.4	0.1783
Mesh × Sample Date	27,108	1.6	0.0486

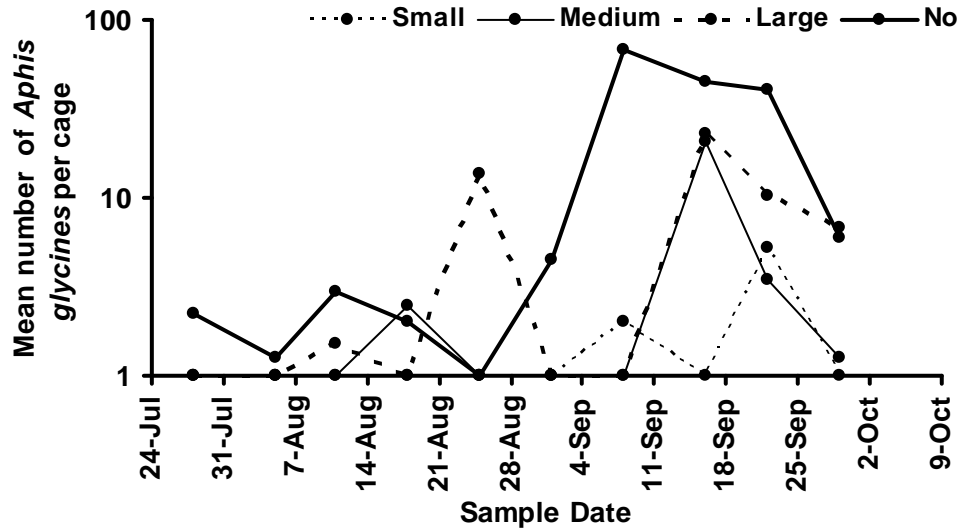


Fig. 22. Mean number of *A. glycines* per cage in small, medium, large, and control cage treatments in uninfested cages.

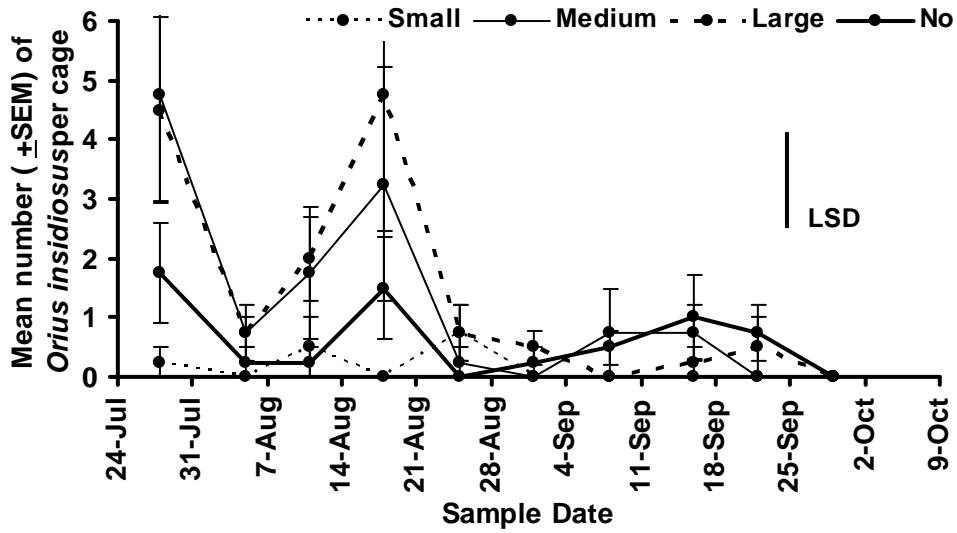


Fig. 23. Mean number of *O. insidiosus* per cage in small, medium, large, and control cage treatments in uninfested cages. LSD=1.8 ($P=0.05$).

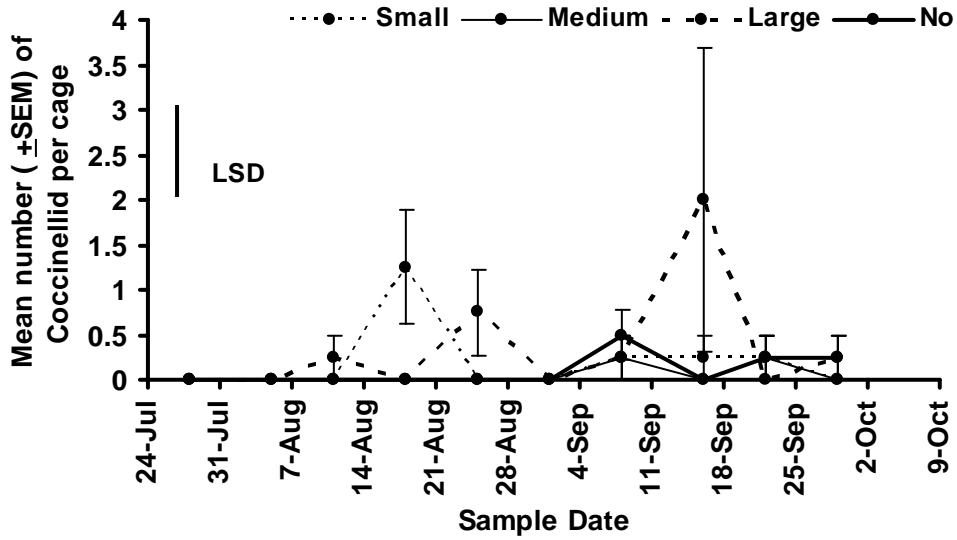


Fig. 24. Mean number of Coccinellid per cage in small, medium, large, and control cage treatments in uninfested cages. LSD=0.9 ($P=0.05$).

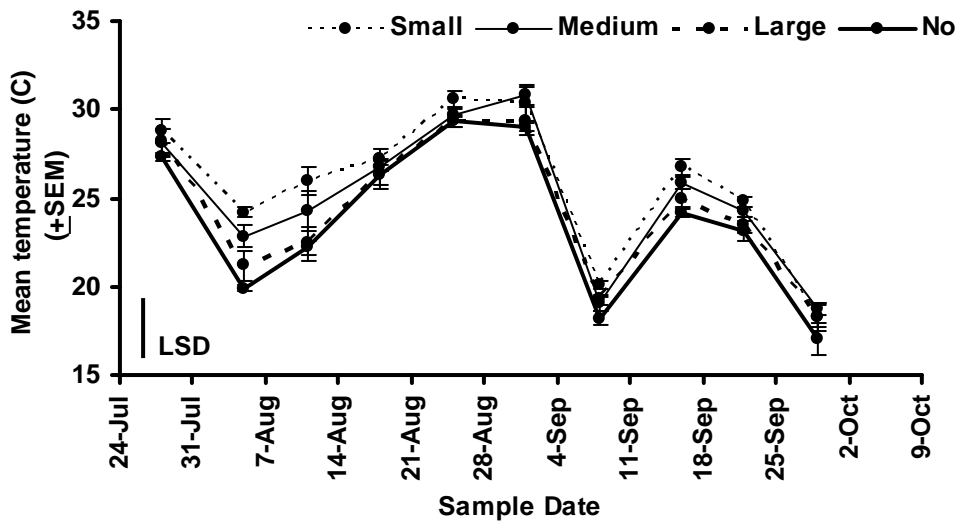


Fig. 25. Mean temperature ($^{\circ}\text{C}$) in small, medium, large, and control cage treatments in uninfested cages. LSD=2.7 ($P=0.05$).

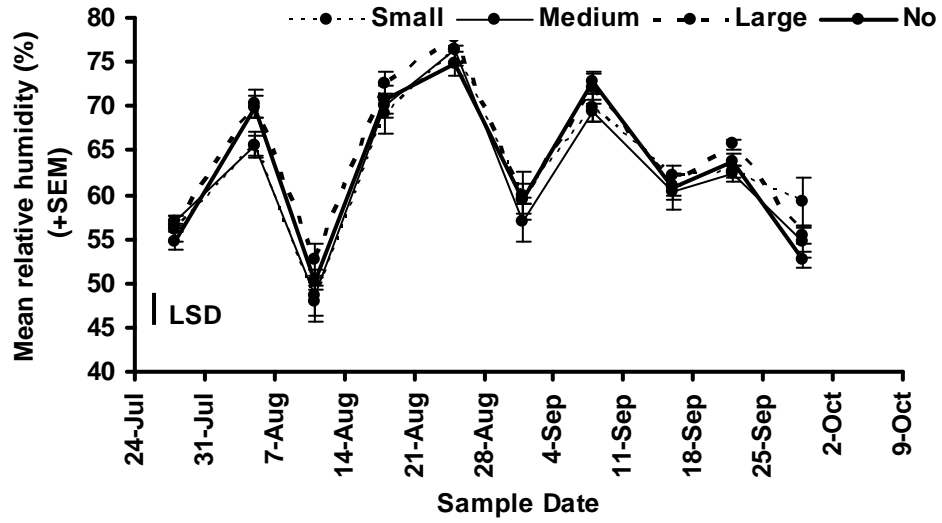


Fig. 26. Mean relative humidity (%) in small, medium, large, and control cage treatments in uninfested cages. LSD=4.1 ($P=0.05$).

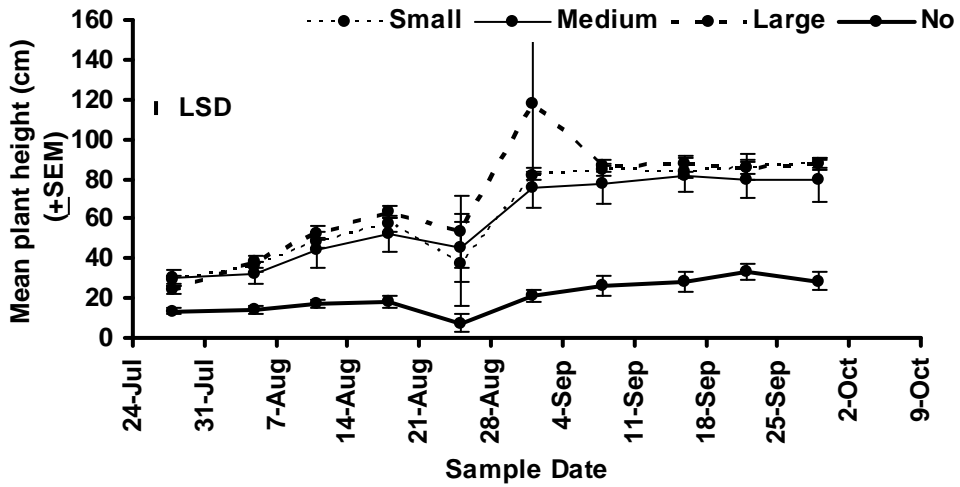


Fig. 27. Mean plant height (cm) in small, medium, large, and control cage treatments in uninfested cages. LSD=8.5 ($P=0.05$).

Discussion

Exclusion of predators by physical barriers followed by observations of the prey population is a method commonly used to assess the importance of natural enemies on a population (Luck et al. 1988). In exclusion cages, Liu et al. (2004) proposed three hypotheses to explain the growth of aphid populations: 1) microclimates may differ and thus affect aphid reproduction or survival; 2) cages may reduce aphid emigration; 3) cages may reduce aphid mortality by excluding natural enemies.

Several exclusion studies have been conducted to evaluate the role of predators in establishment and spread of soybean aphid. Fox et al. (2004) found that cage effects were minimal using temperature, humidity, and plant height measurements; however, aphid populations and predator communities were strongly affected by cage treatments. Foliar-foraging predators prevented soybean aphid population growth and maintained numbers below 10 aphids per plant, while aphid populations in exclusion cages exceeded 200 aphids per plant. When predators were excluded (in previously open cages), small soybean aphid populations increased. Fox et al. (2004) concluded that predators kept soybean aphid populations low in the previously open cages. *Orius insidiosus* adults constituted 61.7% and 55.2% of predators observed in open and frame cages, respectively. When predators had access to previously high soybean aphid populations, aphid populations decreased while predator abundance increased from less than 5 to over 60 predators per m². *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) adults, *O. insidiosus* adults, and *Leucopis* spp. (Diptera: Chamaemyiidae) larvae were the most abundant predators, comprising 22.9, 14.0, and 30.6% of the total predators, respectively. *Harmonia axyridis* populations remained elevated throughout the trial, while *O.*

insidiosus and *Leucopis* spp. population increases were temporary. Fox et al. (2004) concluded that not only were predators capable of keeping soybean aphid populations low, but they also quickly reduced high soybean aphid populations.

The most abundant predators observed during the exclusion study were *O. insidiosus* and several coccinellid species (Table 2). *Orius insidiosus* adults and immatures comprised 39.5% while coccinellid adults and immatures comprised 37.4% of observed predators. Among cages infested on July 21, *O. insidiosus* was present throughout the season while coccinellids were more numerous later in the season. Thus, *O. insidiosus* may be more beneficial in suppressing soybean aphid establishment. Coccinellids, which are known to be attracted to plant volatiles given off by aphid feeding (Zhu and Park 2005) and also stimulated to oviposit by honeydew (Carter and Dixon 1984), may be more beneficial at suppressing already established soybean aphid populations.

In the current study, predator exclusion affected the length of time from aphid infestation until economic threshold was reached. Among July 21 infested cages, economically significant populations of soybean aphid were established two, three, and four and a half weeks after infestation for cages with small, medium and large mesh cages, respectively (Fig. 4). Among August 5 infested cages, economically significant populations were established five and six weeks after infestation for small and medium mesh cages (Fig. 10). July 21 and August 5 infested cages with no mesh never reached the economic threshold (Fig. 4 and Fig. 10). Our results suggest that predatory insects are capable of suppressing soybean aphid populations in Missouri. Data from the

exclusion study, when combined with data from the insecticide study imply that biological control may be all that is needed in Missouri to prevent economic damage.

The rate of increase for soybean aphid populations differed with treatment and infestation date (Table 1). Among cages infested on July 21, soybean aphids in cages with small mesh (excludes all predators) had a significantly higher rate of increase than cages with medium mesh, large mesh, or no mesh . Cages with medium and large mesh had a significantly higher rate of increase compared to cages with no mesh. Among cages infested on August 5, soybean aphids in cages with small and medium mesh had significantly higher rates of increase compared to cages with large mesh and no mesh.

My research suggests that it is possible for large populations of soybean aphids to occur in Missouri and also that those natural enemies already present in Missouri are sufficient to suppress soybean aphid populations. These results are comparable to the study by Venette and Ragsdale (2004) which indicates that southern Missouri would provide a suitable climate for soybean aphid. These results also support current research on predators by Fox et al. (2004, 2005) and Rutledge et al. (2004). What this means for Missouri is that in most years soybean aphid will not be a serious pest.

Predatory insects which should have been excluded based on size were sometimes encountered in exclusion cages. On all mesh sizes, the honeydew on the cage fabric stimulated coccinellid and chrysopid adults to oviposit on the mesh (Carter and Dixon 1984). Young larvae then moved through the mesh, making it difficult to maintain predator free treatments. When this occurred, the number of insects was recorded and the insects removed from the cage.

Cage material can affect soybean aphid emigration, thus affecting soybean aphid populations inside cages. Li-hua and Rui-lu (1993) determined that crowding of adult apterae was primarily responsible for production of alates in soybean aphid. Large numbers of alate aphids were not observed until late August, when populations were already rapidly increasing. Thus, early in the season when alates were few, emigration would likely not be affected by mesh. Later in the season, when populations were crowded and plant health was declining, it is likely emigration was affected by mesh. A decrease in emigration as a result of mesh size would contribute to an increase in soybean aphid populations in cages with small and medium mesh. The effect of decreased emigration is best seen in early infested cages with medium and large mesh (Fig. 3). Soybean aphid populations in cages with medium and large mesh increase to peak in late August, then begin decreasing, and then increase again during the last two sampling periods. This late season increase in soybean aphid population is likely due to alate aphids returning to soybean plants and reproducing. Our observations are similar to those reported in exclusion studies using the Russian wheat aphid by Lee et al. (2005). In cages with small mesh, this late season increase in soybean aphid populations does not occur. This is most likely due to high mortality by fungal infections occurring among the large soybean aphid populations in cages with small mesh. Alate aphids are particularly susceptible to fungal infections and so probably died before returning to soybean plants to reproduce (Nielsen and Hajek 2005).

Although cage material characteristics had little effect on soybean plant growth, they may still have affected soybean aphid population growth. Econet S and Econet L, used in cages with small and medium mesh, reduce available light and airflow. Econet S

reduces airflow by 45% and available light by 9% while Econet L reduces airflow by 5% and available light by 14%. These characteristics could reduce aphid mortality due to rain and wind compared to cages with no mesh. Dixon (1977) concluded that rain and wind had a greater effect on aphid populations than previously thought. Heavy rainfall has been shown to be an important mortality factor in other aphid species such as the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (Shull 1925, Walker et al. 1984), the rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) (Maelzer 1977), the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) (Hughes 1963), and the mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae) (Singh 1982). Heavy rainfall has also been shown to negatively impact the establishment of thrips (Norris et al. 2002).

In the current experiment, plant height in cages without mesh differed significantly from cages with small, medium, and large mesh at all infestation dates. This was due to rodent feeding which occurred in cages with no mesh. Rodent feeding cut the plants off close to the ground resulting in short plants in a later stage of development than other cages. Bird mesh was stretched across the bottom 0.61 m on 16 August; however, the plants were unable to recover and remained shorter for the remainder of the sampling dates. There was little difference between plant heights in cages with small, medium, and large mesh.

Microclimate also differed slightly among treatments. Cages with no mesh had the lowest temperature followed by cages with large, medium, and small mesh with the highest temperature. Mean temperature between mesh treatments varied by $\pm 2^{\circ}\text{C}$ while the mean temperatures in cages over the season ranged from 21°C to 26°C . The optimum

temperatures for soybean aphid development is reported to be between 22 and 27°C (Hirano et al. 1996, McCornack et al. 2004). Above 32°C developmental time increases and survival rate decreases. Weather conditions at the Bradford Research & Extension Center (approximately 4 miles east of research site) report only 6 days in which the high temperature was above 32°C. This suggests that high temperature, and thus a negative impact on soybean aphid development, would be minimal.

Relative humidity differed significantly across treatments during the August infestation only. Mean relative humidity in cages over the season ranged from 61 to 64%. Late season fungal outbreaks were observed in cages with high soybean aphid numbers. These resulted in a blackening of the leaves as the fungus grew on the honeydew covering the leaves. Eventually entire plants were covered and soybean aphid populations subsequently declined. While high relative humidity is known to be conducive to growth of fungal pathogens (Tanada and Kaya 1993), I believe our fungal outbreaks were more the result of high soybean aphid numbers as Nielsen and Hajek (2005) found that levels of fungal infection were strongly associated with aphid density.

Some areas for future research include investigations into the inundative or augmentative release of aphid predators such as *O. insidiosus* and *H. axyridis*, both of which are easily laboratory reared (Isenhour and Yeargan 1981, Koch 2003). Along with inundation and augmentation, the use of selective insecticides toxic to the pest insect and not natural enemies could preserve natural populations. Also, research into soybean aphid life tables and key factor analysis would greatly improve our understanding of soybean aphid biology. Lastly, investigation into the role and importance of intraguild

predation on suppression of soybean aphid populations could aid in our understanding of predator-prey interactions.

CHAPTER IV

PREDATOR-PREY INTERACTIONS INVOLVING THE SOYBEAN APHID (HEMIPTERA: APHIDIDAE) IN MISSOURI.

ABSTRACT Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is found in all soybean, *Glycine max* L., growing regions within Missouri. Despite soybean aphid's widespread distribution in Missouri, it has reached economic threshold in very few soybean fields, possibly due to predatory insects such as *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Field experiments were performed over two years to examine the impact insecticide applications aimed at bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), have on soybean aphid populations and their natural enemies. Insecticide treatments consisted of an early spray, a late spray, an early and late spray, and a non-sprayed control. Insecticide applications were based on soybean growth stages. In 2003, early and late spray applications were made at V1 and R2 plant growth stage, respectively. In 2004, early and late spray applications were made at V6 and R2-R3 plant growth stage, respectively. Additionally, an unsprayed control was included as a treatment. Whole plant counts of thrips, *O. insidiosus*, and soybean aphids from 10 randomly selected plants were taken at 7-10-d intervals. Soybean aphid populations varied considerably between 2003 and 2004, with 2003 having larger soybean aphid populations. During 2003 soybean aphid populations remained near zero until the end of July, when populations rapidly increased and then began decreasing until the end of sampling. 2004 soybean aphid populations remained near zero throughout the season. *Orius insidiosus* populations were present throughout the season, peaking during late July. Thrips, a principal prey item for *O. insidiosus*, were also present throughout the

season. No resurgence of soybean aphid populations was observed following insecticide applications.

KEY WORDS *Aphis glycines*, *Orius insidiosus*, Predator-prey interactions, Carbaryl

Introduction

Soybean, *Glycine max* L., protection can be dependent upon broad-spectrum chemical insecticides which are toxic to pests as well as natural enemies. Natural enemies can be exposed to insecticides directly by spray droplets, indirectly as residues on plant surfaces, or when feeding on contaminated water droplets, nectar, or honeydew (Banken and Stark 1998). Thus, insecticide exposure of predators and parasitoids is probable in soybean that is sprayed with insecticide.

Disruption of natural enemies or parasitoid populations by an insecticide application often results in increasing pest populations (resurgence) or an increase in secondary pests (replacement). Insecticide applications affect insect populations (prey and predator) within an agroecosystem directly and indirectly. The most obvious direct effect is increased mortality. Mortality of pest and natural enemy populations may differ due to differences in the route of exposure and the susceptibility of the insect to the insecticide. Following insecticide application, pest and natural enemy populations may recover differently based on the route of exposure and their reproductive rates (Hardin et al. 1995).

In the United States, soybean is the number two (29%) agricultural crop planted by area (SoyStatsTM 2005). In 2004, 30.4 million hectares were planted to soybean in the United States with a value of \$17.7 billion. Missouri planted 2 million hectares to soybean in 2004, more than any other crop (SoyStatsTM 2005). Before the discovery of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in 2000 (Wedberg 2000), bean leaf beetle, *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae), was the most important soybean pest in the North Central United States. Bean leaf beetle is

the main vector of bean pod mottle virus (BPMV) in the North Central United States (Giesler et al. 2002). Severity of symptoms and yield loss is dependent on the virus strain, soybean variety, and time of infection with earlier infections causing greater yield loss (Giesler et al. 2002).

Current recommendations for suppression of bean leaf beetle, and subsequently BPMV, consist of an early season and mid season insecticide application (Krell et al. 2004). Greenbook (2006) lists 36 insecticides currently registered for use against bean leaf beetle. However, the introduction of soybean aphid has complicated conventional bean leaf beetle suppression. In Missouri, where soybean aphid is usually a secondary pest, insecticide applications for bean leaf beetle could allow soybean aphid to replace bean leaf beetle and become a major pest.

Insecticide applications affect all insects within a field, not only the pest insect. The mortality of predatory insects within an agricultural system can contribute to pest resurgence. For this reason, populations of a key predatory insect, *Orius insidiosus* Say (Hemiptera: Anthocoridae), were also sampled during the experiment. In conjunction with direct mortality, predatory insect numbers can be reduced following insecticide application due to the mortality of their prey insect. For this reason, the principal prey insect of *O. insidiosus*, thrips, were also sampled.

The carbamate insecticide Sevin® brand XLR Plus (carbaryl) is frequently used for suppression of BLB populations. However, its effect on soybean aphid populations and their natural enemies is not known. The current study was aimed at determining what effect this insecticide will have on populations of soybean aphid and their natural enemies.

Materials and Methods

Experimental Design. Studies were conducted over a two year period (2003-2004) at two locations in Boone County in central Missouri: The University of Missouri, South Farms (92° 17' W, 38° 14' N; elevation \approx 813') and the University of Missouri, Bradford Research and Extension Center (BREC) (92° 17' W, 38° 53' N; elevation \approx 893'), both East of Columbia.

During 2003, fields were planted on 28 May with soybean cultivar 93B85 (Pioneer Hi-Bred International, Inc., Des Moines, IA). In 2004, fields were planted on 2 June (South Farm) and 4 June (BREC) with soybean cultivar DKB 38-523 (Monsanto Company, St. Louis, MO). Cultivar varied by year depending on availability. Seed was planted at a rate of 473,595 seeds per ha in 76-cm rows using a John Deere tractor model 1520 with a John Deere planter model 7000 at both locations. Plots were 9 m \times 9 m with approximately 4,000 plants per plot. In 2003, the experiment was set up in a 2 \times 4 factorial (infestation date \times insecticide application) arrangement of a randomized complete block design and with repeated measures replicated four times. In 2004, the experiment was a single factor experiment (insecticide application) replicated four times in a randomized complete block design. Only one infestation date was used in 2004 because 2003 data indicated there was no difference between the infestation dates.

Soybean aphids were collected from field populations in Columbia, Missouri during the summer of 2002 and reared on soybean plants under greenhouse conditions (L:D 16:8, 25°C \pm 2°C). New plants were supplied weekly and infested by placing an

infested leaflet on the new leaves. Field infestations were accomplished by attaching aphid infested leaves (10-20 aphids per leaf, colony reared) onto the first trifoliolate. Each 9 m row received 15 leaf cuttings (one every 0.6 m), or approximately 1800 to 3600 aphids per plot. There were two different infestation dates in 2003, early (27 June; V3) and late (1 August; R3). In 2004 all blocks were infested at the same time (9 August; R3).

Insecticide treatments involved either plots that were treated early with one insecticide application, late with one insecticide application, early and late insecticide applications (two total applications), or plots that received no insecticide applications (control). In 2003 the early spray treatment received an insecticide spray on 17 June, when plants were approximately three weeks of age (V1). The late spray treatment received an insecticide spray on 24 July, when plants were approximately eight weeks of age (R2). The treatment with both an early and late spray received an insecticide spray on 17 June and 24 July, at three weeks and eight weeks of age. Control plots received no insecticide treatments. In 2004, early spray treatment received an insecticide spray on 13 July, when plants were approximately six weeks of age (V6). Late spray received an insecticide spray on 2 August, when plants were approximately eight weeks of age (R2-R3). The treatment with both an early and late spray received an insecticide spray on 13 July and 2 August, at six weeks and eight weeks of age. Control plots received no insecticide treatments. Insecticide application consisted of carbaryl (Sevin® brand XLR Plus, Bayer Crop Protection, Kansas City, MO) and was applied as a foliar spray at the recommended rate of 1150 g (AI)/ha. Applications were made with a CO₂ backpack

sprayer equipped with four 8001VS stainless steel spray tips and calibrated to deliver 140 liters/ha at 20 psi.

Weeds in all plots and alleys were controlled using glyphosate (Roundup WeatherMAX®, Monsanto Company, St. Louis, MO), which was applied at a rate of 864 g (AI)/ha. In 2003, glyphosate applications were made on 12 June and 29 June at BREC and 2 July at South Farm. In 2004, glyphosate applications were made on 14 June and 23 July at BREC and 11 June and 15 July at South Farm. Alleys were also disced to reduce weeds. In 2003, alleys were disced on 17 June and 30 June at BREC and 16 June and 7 July at South Farm. In 2004, alleys were disced on 15 July at South Farm.

Sampling Methods. Within each 9 m × 9 m plot, ten whole plants were collected at random at weekly intervals from mid June to early September (11 June to 3 September in 2003 and 16 June to 3 September in 2004). Plants were collected on eleven dates at South Farm in 2003 and nine dates at BREC in 2003. In 2004, plants were collected on twelve dates at both locations. Individual plants were cut approximately 25 cm above soil level and a plastic bag maneuvered under the plant. The bag was then pulled up and over the top of the plant to reduce insect escape. Plant growth stage was recorded each sampling as described by Fehr et al. (1971). Collected plants were stored at 4°C until processing the following day.

Processing of Insect Samples. Plants were removed from bags and rinsed with water to remove insects. Bags were also rinsed to remove any insects stuck to the bag. Rinsate was then passed through two soil sieves; U.S.A. Standard Sieve Series Sieve No. 20 (850 µm) to capture large insects and leaf parts, and U.S.A. Standard Sieve Series Sieve No. 230 (63 µm) to catch all other insects. Recovered insects were placed in

specimen cups containing 95% ethanol (The Kendall Company, Mansfield, Massachusetts) until they could be counted and identified.

After storage, samples were processed using the following procedure: the contents of each cup were emptied into U.S. Standard Sieve Series No. 230 (63 μm). The interior of the specimen cup was thoroughly rinsed with water to remove any remaining insects. To separate insects from residual plant material, the following procedure was used. Each sample was placed in a 2 M magnesium sulfate solution held in a 100 ml beaker. The sample mixture was agitated by swirling to separate any clumps of plant material. The sample was then poured into a separatory funnel. Beaker and funnel sides were rinsed with the magnesium sulfate solution to remove entire sample. The sample was left to separate for 15-20 minutes. Settled debris was poured into a beaker and disposed of, the supernatant was removed into a separate beaker. The supernatant was filtered through gridded (5 mm \times 5 mm) filter paper (9.0 cm diameter, Whatman® #1 qualitative, Florham Park, New Jersey) using a Buchner funnel. The beaker was rinsed with tap water to collect the entire sample. Filter paper with the sample was placed in a petri dish lid (100 \times 15 mm standard, Fisherbrand®, Hampton, New Hampshire) and the insects counted. Insect densities were determined under 50 \times magnification using a microscope (Wild M5A, Heerbrugg, Switzerland).

Statistical Analysis. Insect densities from each treatment and over the sampling season were square root transformed and analyzed with the SAS mixed procedure (SAS 2001) (Appendix 2). The basic analysis of variance was a randomized complete block design split plot in time. Each year and each location were analyzed separately. The linear statistical model contains the effect of early, late, and the interaction of early \times late

spray. The sub-plot contains the effect of date and all possible interactions with the main plot effects. Differences between means were determined using Fisher's least significant difference (LSD). Analysis of variance tables contain results from transformed data while graphs are of untransformed data.

Results

***Orius insidiosus* sampling.** *Orius insidiosus* was detected in low numbers throughout the season, gradually increasing until late July. Peak *O. insidiosus* densities occurred during late July to early August of each year. *Orius insidiosus* populations were affected by sample date at all locations during both years ($P < 0.0001$). During 2003, *O. insidiosus* populations were affected by the early spray \times sample date ($P < 0.0001$) at South Farm, while at BREC *O. insidiosus* populations were affected by the late spray \times sample date ($P < 0.05$). During 2004, *O. insidiosus* populations were unaffected by any treatment.

2003. *Orius insidiosus* populations at South Farm, early (Fig. 28) and late (Fig. 30) infested, were affected by the early spray \times sample date ($P < 0.0001$). Populations at BREC, early (Fig. 29) and late (Fig. 31) infested, were affected by the late spray \times sample date ($P < 0.05$).

2004. *Orius insidiosus* populations at South Farm (Fig. 32) and BREC (Fig. 33) were unaffected by any treatment.

Thrips sampling. Thrips were detected in high numbers throughout the season. Peaks in thrips densities occurred in early July at South Farm in 2003 and mid July at

South Farm in 2004. Thrips populations at BREC in 2003 and 2004 did not have a well defined peak. In general, there were more thrips observed at South Farm than BREC in 2003. Thrips populations were affected by sample date at all locations during both years ($P < 0.0001$). During 2003, populations were affected by early spray \times sample date ($P < 0.0001$) at both locations. During 2004, populations were affected by early spray \times sample date ($P < 0.05$) at South Farm while populations at BREC were unaffected by any treatment.

2003. Thrips populations at South Farm (Figs. 28 and 30) were affected by the early spray \times sample date interaction ($P < 0.0001$). Both early and late infested populations exhibited peak thrips densities during early July, two weeks after the early insecticide application. Thrips populations in early and early/late insecticide treatments were lower than those of the late and no insecticide treatments one and two weeks following application. By three weeks following application, populations in all treatments were again similar. Thrips populations at BREC (Figs. 29 and 31), both early and late infested, were again affected by the early spray \times sample date interaction ($P < 0.0001$). Early and early/late treatment populations were lower than late and no insecticide treatment populations two and three weeks following application. By four weeks following application, populations in all treatments were again similar.

2004. Thrips populations at South Farm (Fig. 32) were affected by the early spray \times sample date interaction ($P < 0.05$). Populations at BREC (Fig. 33) were unaffected by any treatment.

Aphid Sampling. Soybean aphid was detected in very low numbers until early August, when populations began to rise. Peak soybean aphid densities occurred during

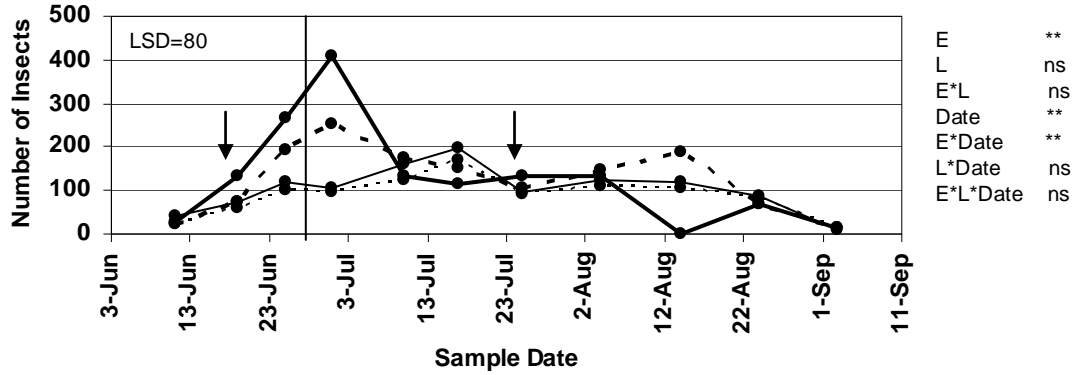
mid to late August of each year. Soybean aphid populations varied considerably between 2003 and 2004, with 2003 having higher numbers of soybean aphid. In general, more soybean aphids were observed at the South Farm location than the BREC location. Soybean aphid populations were affected by sample date at all locations during both years ($P < 0.0001$). Populations at BREC in 2004 were affected by early spray \times sample date ($P < 0.05$); all other soybean aphid populations were unaffected by any treatment.

2003. Soybean aphid populations at South Farm, early (Fig. 28) and late (Fig. 30) infested, and BREC, early (Fig. 29) and late (Fig. 31) infested, were not affected by any of the treatments. Populations were still near zero when the first insecticide application was made and so no impact was observed. Populations were low during the second insecticide application, and peaked two weeks later. However; this was not the result of resurgence because increases were observed in all treatments. It is more likely this was the result of immigration from nearby fields.

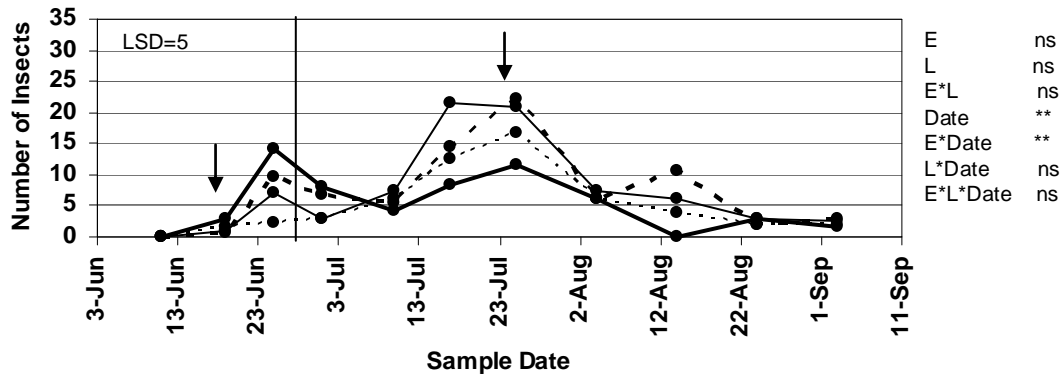
2004. Soybean aphid populations were significantly lower in 2004 than 2003. Populations at South Farm (Fig. 32) were unaffected by any treatment while populations at BREC (Fig. 33) were affected by the early spray \times sample date ($P < 0.05$). This variability may be due to the extremely low numbers of soybean aphid observed during 2004.

Early Aphid Infestation

2003 South Farm Total Thrips



2003 South Farm Total *O. insidiosus*



2003 South Farm Aphids

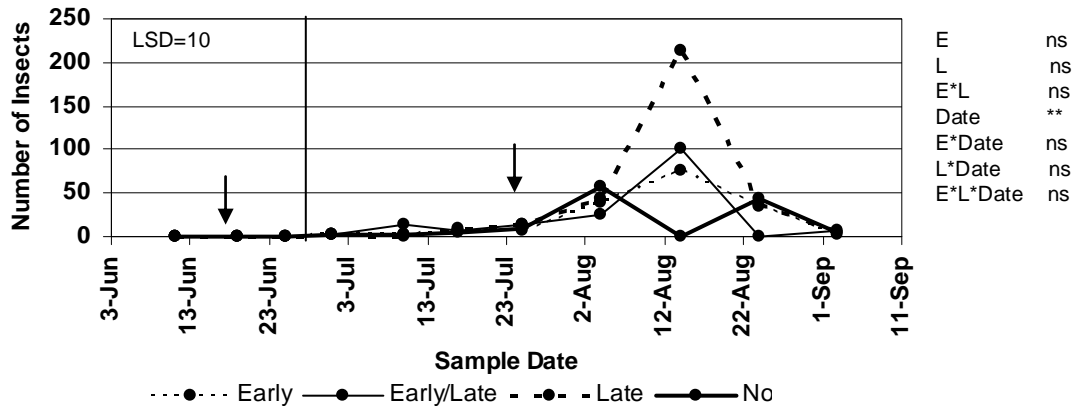
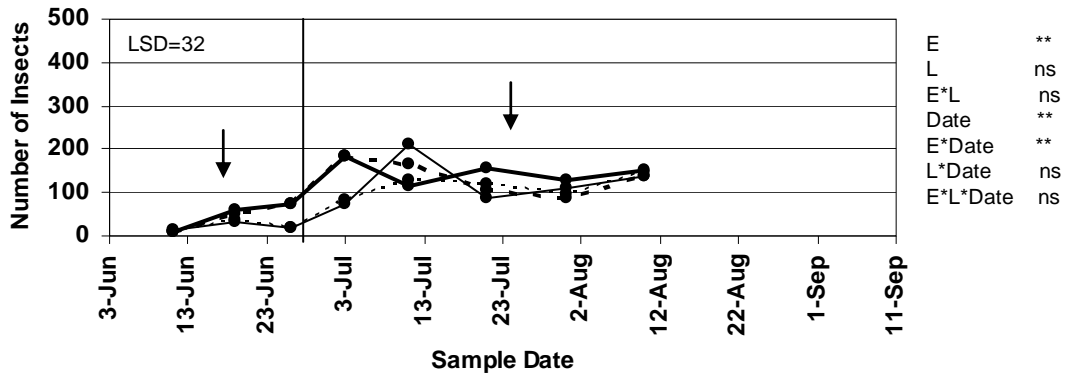


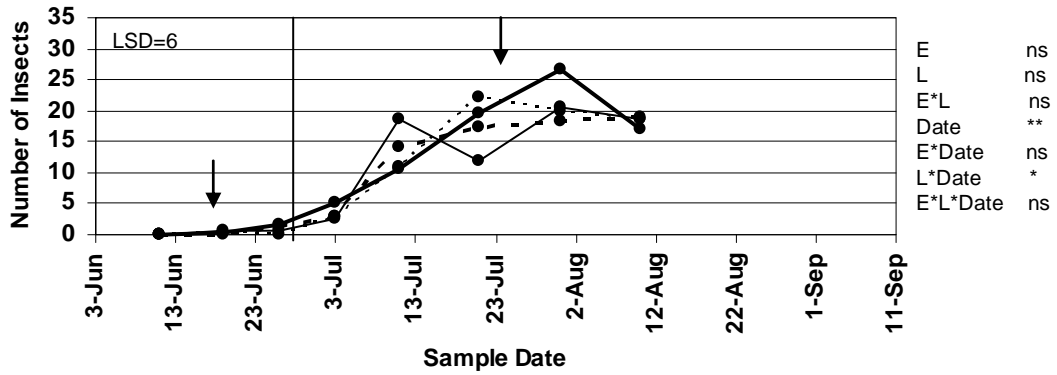
Fig. 28. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of 93B85 among early infested plots at South Farm in Boone Co., Missouri, in 2003. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

Early Aphid Infestation

2003 Bradford Total Thrips



2003 Bradford Total *O. insidiosus*



2003 Bradford Aphids

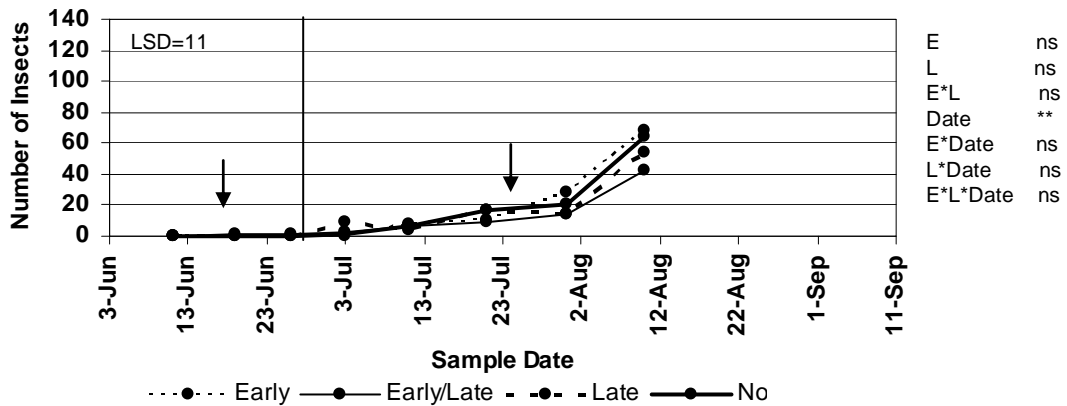
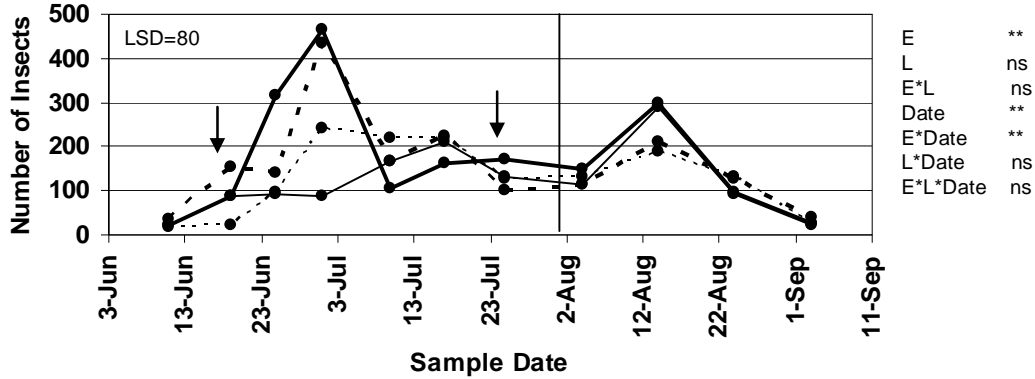


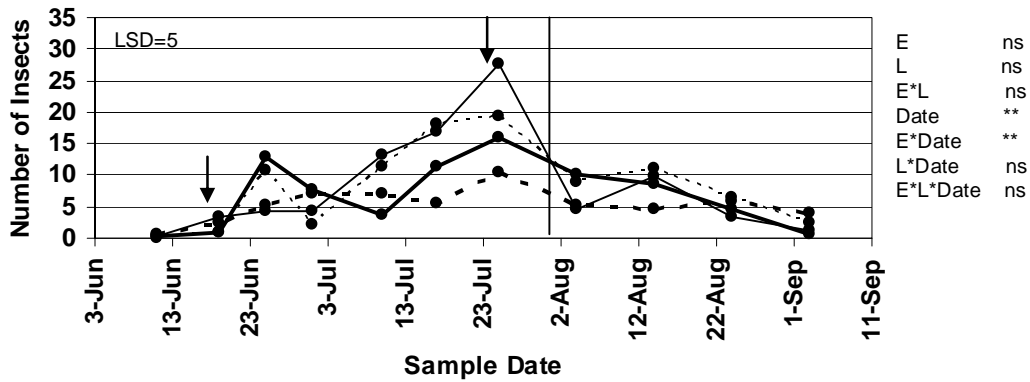
Fig. 29. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of 93B85 among early infested plots at BREC in Boone Co., Missouri, in 2003. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

Late Aphid Infestation

2003 South Farm Total Thrips



2003 South Farm Total *O. insidiosus*



2003 South Farm Aphids

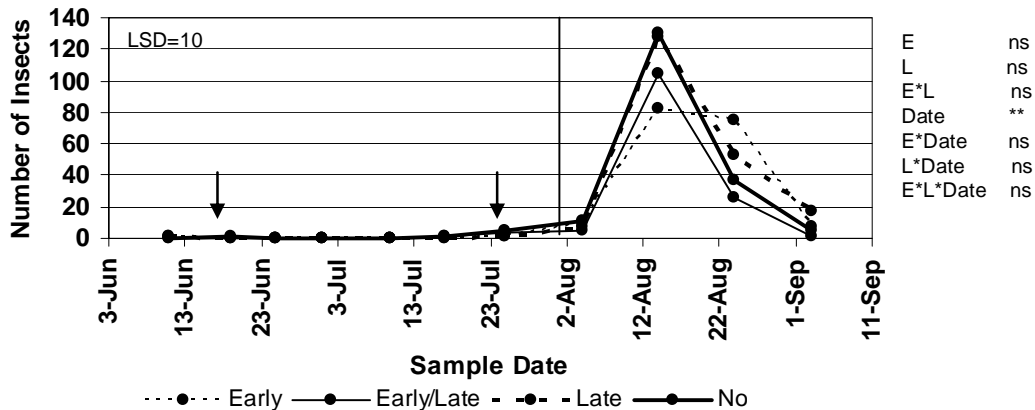
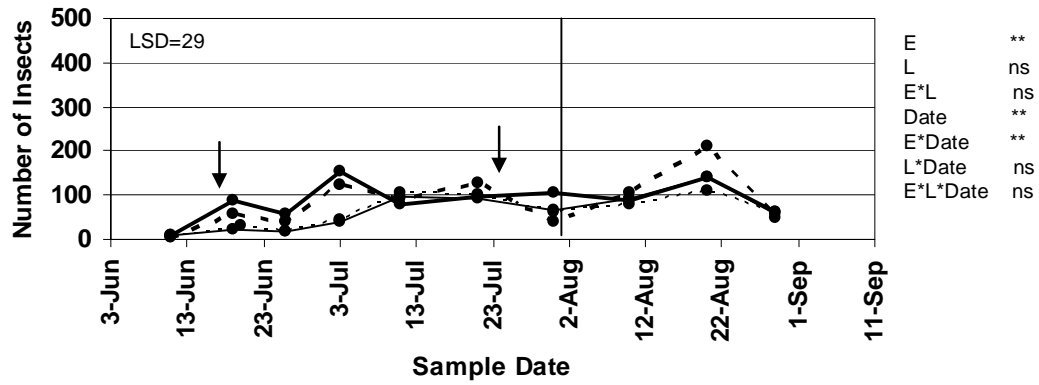


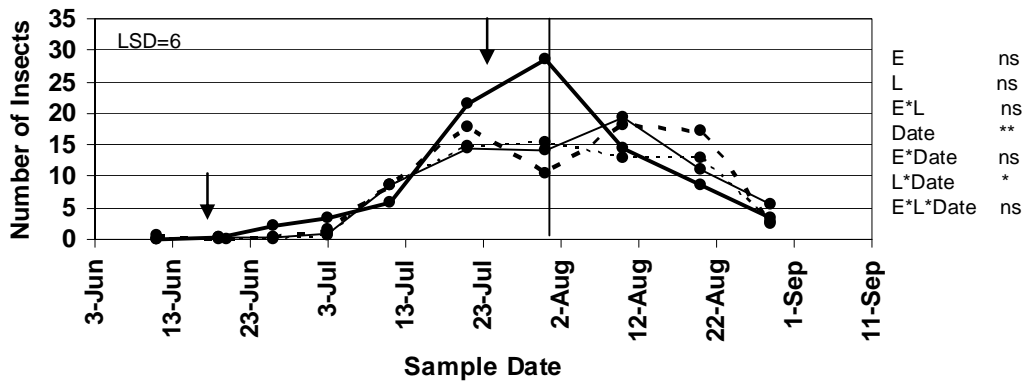
Fig. 30. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of 93B85 among late infested plots at South Farm in Boone Co., Missouri, in 2003. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

Late Aphid Infestation

2003 Bradford Total Thrips



2003 Bradford Total *O. insidiosus*



2003 Bradford Aphids

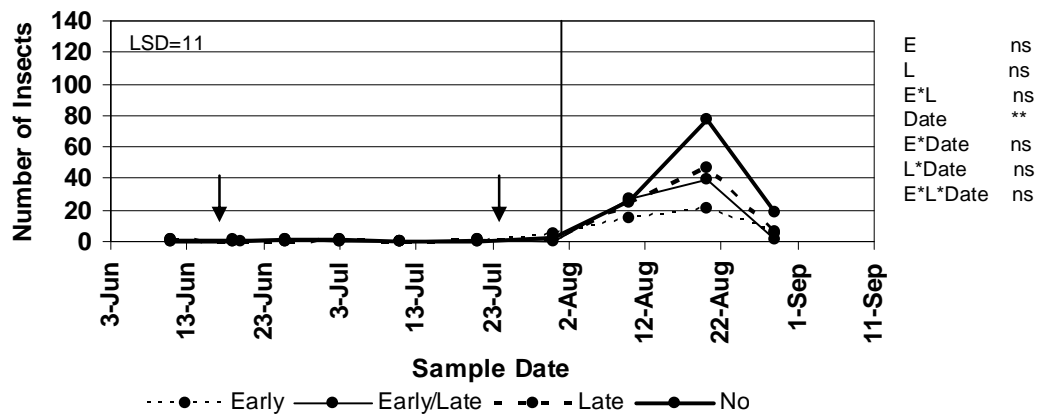
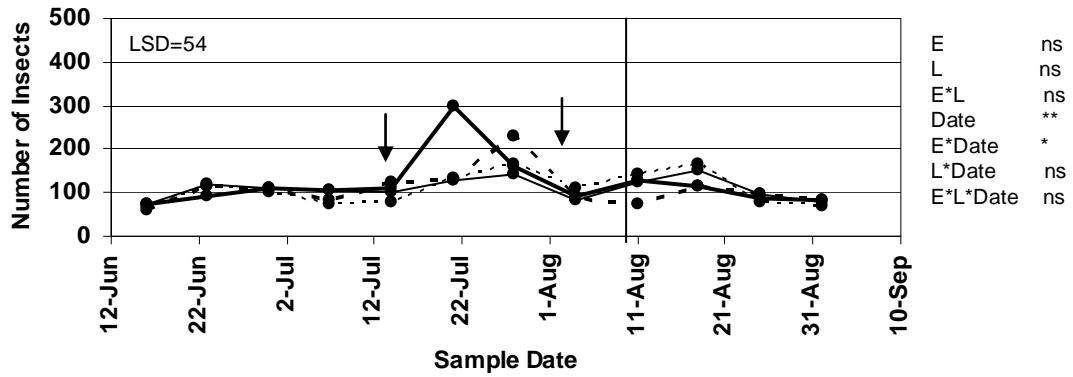
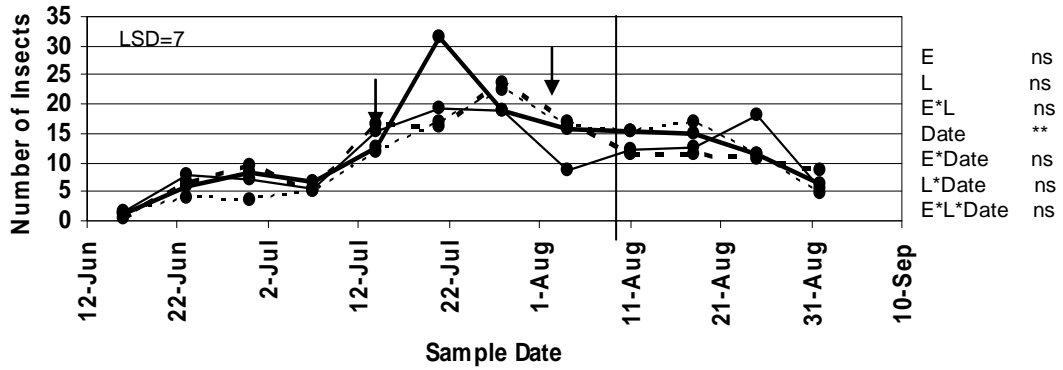


Fig. 31. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of 93B85 among late infested plots at BREC in Boone Co., Missouri, in 2003. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

2004 South Farm Total Thrips



2004 South Farm Total *O. insidiosus*



2004 South Farm Aphids

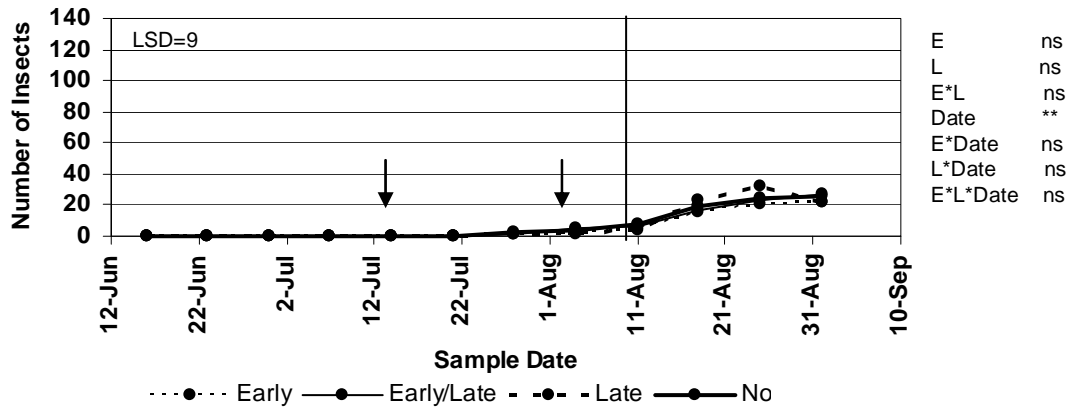
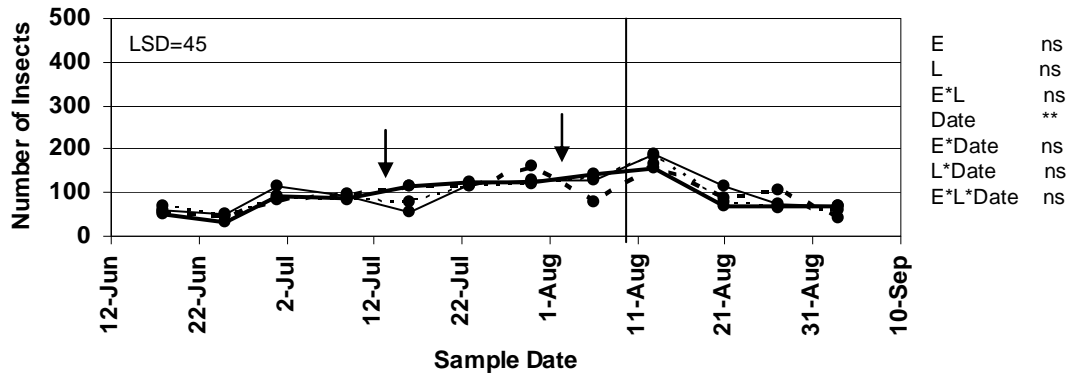
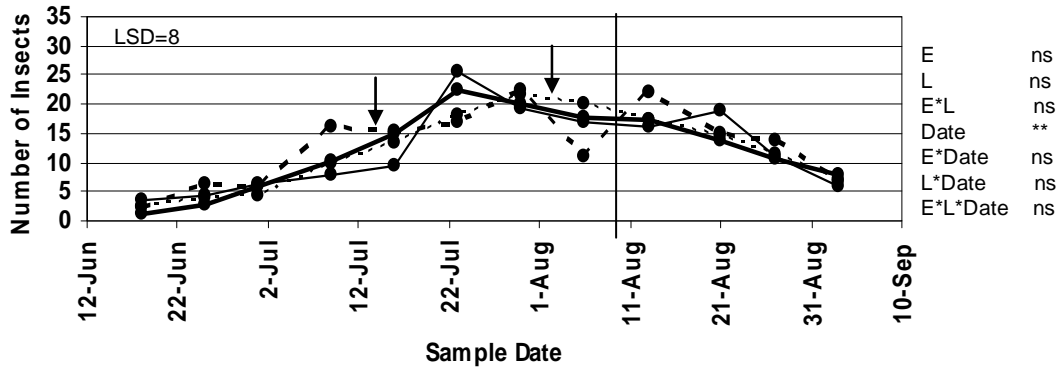


Fig. 32. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of DKB 38-523 among plots at South Farm in Boone Co., Missouri, in 2004. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

2004 Bradford Total Thrips



2004 Bradford Total *O. insidiosus*



2004 Bradford Aphids

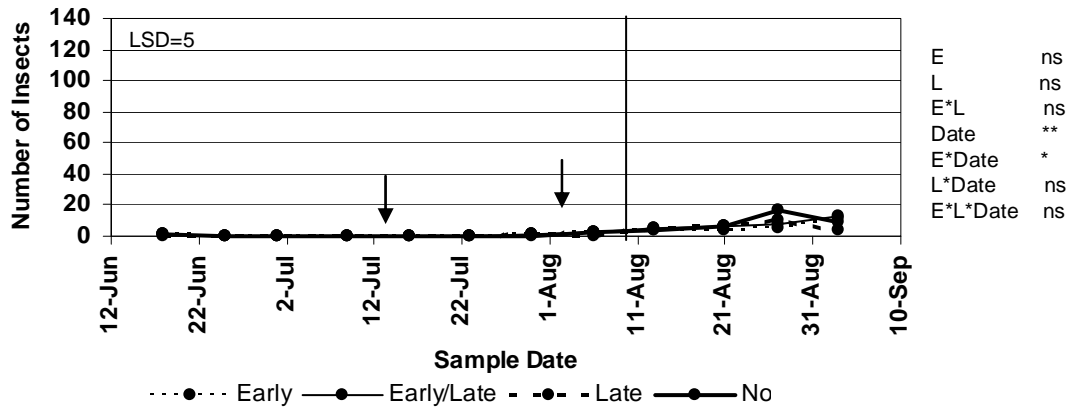


Fig. 33. Mean densities of thrips spp., *O. insidiosus* and soybean aphid per 10 plants in early, early/late, late and control treated cultivars of DKB 38-523 among plots at BREC in Boone Co., Missouri, in 2004. Arrows indicate insecticide application to the treated plots. Vertical line indicates aphid infestation date.

Discussion

Previous research has shown that in agricultural systems, aphid populations can increase dramatically following an insecticide application (Natarajan et al. 1986a, b, Rangarajan et al. 1986, Hardin et al. 1995). A principle reason for these increases in aphid populations is the mortality of predatory insects. In the current study, no resurgence of soybean aphid populations was observed following application of Sevin® XLR Plus. One possible reason for the lack of aphid resurgence is that Sevin® XLR Plus may not significantly disrupt the predator-prey dynamic of the agricultural system. Carbaryl is not registered for control of soybean aphid and none of the aphid populations observed during the study were affected by carbaryl applications. Carbaryl is used for control of bean leaf beetle in Missouri because it is less toxic to predatory insects (W. Bailey, personal communication). However, carbaryl is toxic to *H. axyridis* and *O. insidiosus* at higher doses than was used in this study (Michaud and Grant 2003).

O. insidiosus, the principal predator investigated during this study, was unaffected by the early or late spray during either year. Their primary prey item, thrips were affected by insecticide sprays. The removal of prey items could result in a reduction in *O. insidiosus* populations either thru mortality or emigration. However, the presence of a secondary prey item, soybean aphid, which was not affected by insecticide sprays could contribute to the food supply for *O. insidiosus*. Thus, during periods of low prey numbers following an insecticide application, soybean aphid could serve to maintain *O. insidiosus* populations.

Soybean aphid densities did not reach economic threshold at any location during either year. During 2003, soybean aphid infestations were carried out approximately two weeks after the first insecticide application when plants were at V3. Infestations were also carried out approximately one week following the late insecticide application when plants were at R3. During 2004, infestations were carried out one week following the late insecticide application when plants were at R3. Each 9 m × 9 m plot was infested with between 1800 to 3600 aphids. Despite infestations, the highest density of soybean aphid during the study was approximately 20 aphids per plant. This is in contrast to the extremely high soybean aphid populations observed in exclusion cages, which were infested at a similar plant growth stage using the same infestation technique and the same soybean variety. There are many possible reasons why soybean aphid densities never reached economic threshold.

The most likely reason for reduced soybean aphid establishment is the presence of natural enemies. Naturally occurring predatory insects such as *O. insidiosus* are present early in the season and may aid in reducing establishment of pest populations (Rutledge et al. 2004). This is most likely why a large increase in soybean aphid numbers was not observed immediately following infestation. Soybean aphid populations only began increasing in mid August, after *O. insidiosus* populations began decreasing. This indicates that, under the infestation levels in this study, *O. insidiosus* and other natural enemies were capable of reducing soybean aphid establishment and prevent resurgence following insecticide application.

Another possibility for reduced soybean aphid establishment is that the soybean variety used was resistant to soybean aphid. This is unlikely because the varieties used

during 2003 and 2004 were commonly used varieties and were not bred for resistance to soybean aphid. Also, high populations of soybean aphid were produced on the same variety used during 2004 in an exclusion cage study. Another possibility is that the infestations were unsuccessful. This is also unlikely since such a large number of aphids were used for infestation and also because the same technique was used later to infest exclusion cages which subsequently produced high populations of soybean aphid.

Plant nutrition may also have affected soybean aphid establishment. Soybean aphid has its highest net reproductive rate and lowest mortality on three week old plants (Van Den Berg et al. 1997). The early infestation occurred when plants were four weeks old and the late infestation occurred when plants were eight weeks old. Thus, early infestations would have higher net reproductive rates and less mortality than late infestations. However, an increase in soybean aphid populations was not observed following the early infestation. Instead, low numbers of soybean aphid were observed until mid August. During 2003, Missouri was experiencing a drought and it is possible that during mid August plants were becoming potassium deficient due to the drought. Soybean aphid has a higher net reproductive rate, intrinsic rate of increase, and finite rate of increase on potassium deficient leaves compared with healthy leaves (Myers et al. 2005b). *Orius insidiosus* populations began declining in late July and soybean aphid populations began increasing shortly after. It is likely that a combination of decreasing predator population and potassium deficient soybean plants allowed soybean aphid populations to increase.

Our data suggests that the soybean aphid will not be an economic problem in Missouri in most years. The presence of a principal predator early in the season, low

numbers of immigrating aphids, and the use of insecticides not toxic to predators act together to suppress soybean aphid populations below threshold. However, changes in any of these conditions could result in changes in soybean aphid pest status in Missouri.

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Appendix 1.

```
data one; infile 'g:\2004 exclusion.csv' dsd firstobs=3 missover;
input
Plot$ Mesh$ Infest$ Date DAI WAI Rep pRH Temp p1-p6 junk APH V R Aphids
Thrips OA OI LaceA LaceL LaceE Spider Fleab
CocA CocL CocP CocE Tiger SyrA SyrP SyrL Velvet
BigEye Ground Rove AphidM ;

ot=sqrt (sum(of oa oi)+1);
coct=sqrt (sum(of coca cocl)+1);
aph=mean(of p1-p6);
taphids=sqrt(aphids+1);
syrt=sqrt (sum(of syra syrps)+1);
if mesh='Large' then m='L';
if mesh='Medium' then m='M';
if mesh='Small' then m='S';
if mesh='No' then m='N';
if infest='Early' then i='E';
if infest='Middle' then i='M';
if infest='Late' then i='L';
if infest='No' then i='N';

/*
proc print;
var Plot Mesh Infest Wai Rep pRH Temp APH V R Aphids Thrips
SyrA SyrP SyrL
ot
coct;

data two; set one;
if WAI<5;
proc glm; classes rep mesh infest WAI;
*model Aphids Taphids=rep mesh|infest|WAI rep(mesh infest);
model WAI APH Aphids Thrips Syrt ot coct=rep mesh|infest|WAI rep(mesh infest);
test h= rep mesh|infest e=rep(mesh infest);
means mesh|infest|WAI;
lsmeans mesh|infest/s p e=rep(mesh infest);
lsmeans mesh|infest|WAI/s p ;
*/

data two; set one;
if WAI<9;
if infest='Late' then delete;

/*
```

```

data two; set one;
if WAI<5;
*/

/*
proc glm; classes rep mesh infest WAI;
*model Aphids Taphids=rep mesh|infest|WAI rep(mesh infest);
model WAI APH Aphids Thrips Syrt ot coct=rep mesh|infest|WAI rep(mesh infest);
test h= rep mesh|infest e=rep(mesh infest);
means mesh|infest|WAI;
lsmeans mesh|infest/s p e=rep(mesh infest);
lsmeans mesh|infest|WAI/s p ;
*/

proc sort; by i;
/*
proc mixed; by i; classes rep m WAI;
model aph = rep m|WAI ;
*random rep ;
repeated wai/sub=rep(m) type=cs;
lsmeans m|WAI/pdiff ;
/*

/*
proc mixed; classes rep m i WAI;
model temp = rep m|i|WAI ;
*random rep ;
repeated wai/sub=rep(m i) type=cs;
lsmeans m|i|WAI/pdiff ;
*/

/*
proc mixed; classes rep m i WAI;
model prh = rep m|i|WAI ;
*random rep ;
repeated wai/sub=rep(m i) type=cs;
lsmeans m|i|WAI/pdiff ;
*/

/*
proc mixed; classes rep m i WAI;
model taphids = rep m|i|WAI ;
*random rep ;
repeated wai/sub=rep(m i) type=cs;
lsmeans m|i|WAI/pdiff ;
*/

```

```
/*  
proc mixed; classes rep m i WAI;  
model ot = rep m|i|WAI ;  
*random rep ;  
repeated wai/sub=rep(m i) type=cs;  
lsmeans m|i|WAI/pdiff ;  
*/
```

```
proc mixed; classes rep m i WAI;  
model coct = rep m|i|WAI ;  
*random rep ;  
repeated wai/sub=rep(m i) type=cs;  
lsmeans m|i|WAI/pdiff ;
```

```
/*  
proc mixed; classes rep m i WAI;  
model syrt = rep m|i|WAI ;  
*random rep ;  
repeated wai/sub=rep(m i) type=cs;  
lsmeans m|i|WAI/pdiff ;  
*/
```

```
run;
```

Appendix 2.

Options ls=100 ps=70;

Data one;

Infile 'G:\2003 sa.csv' dsd firstobs=2 missover;

Input Loc Sam Spray\$ Infest\$ Date Rep TA TY TotT OA OY TotO Aphids Other;

stott=sqrt (tott+1);

stoto=sqrt (toto+1);

saphids=sqrt (aphids+1);

if date > 8 then delete;

if loc=2 and rep=1 then delete;

if infest=late then delete;

e=1; l=1;

if spray='Early' then do;l=0; e=1; end;

if spray='Late' then do; l=1; e=0; end;

if spray='No' then do; l=0; e=0; end;

Proc print; proc sort; by loc;

Proc mixed; by loc; class rep e l date;

model stott = e||date;

random rep rep(e l);

repeated date/sub=rep(e l) type=cs;

lsmeans e||date/pdiff;

run;

Proc mixed; by loc; class rep e l date;

model stoto = e||date;

random rep rep(e l);

repeated date/sub=rep(e l) type=cs;

lsmeans e||date/pdiff;

run;

Proc mixed; by loc; class rep e l date;

model saphids = e||date;

random rep rep(e l);

repeated date/sub=rep(e l) type=cs;

lsmeans e||date/pdiff;

run;

Data two;

Infile 'G:\2003 sa.csv' dsd firstobs=2 missover;

Input Loc Sam Spray\$ Infest\$ Date Rep TA TY TotT OA OY TotO Aphids Other;

stott=sqrt (tott+1);

stoto=sqrt (toto+1);

saphids=sqrt (aphids+1);

if date > 8 then delete;

if loc=2 and rep=1 then delete;

if infest=early then delete;

e=1; l=1;

if spray='Early' then do;l=0; e=1; end;

```

if spray='Late' then do; l=1; e=0; end;
if spray='No' then do; l=0; e=0; end;
Proc print; proc sort; by loc;
Proc mixed; by loc; class rep e l date;
model stott = e||date;
random rep rep(e l);
repeated date/sub=rep(e l) type=cs;
lsmeans e||date/pdiff;
run;
Proc mixed; by loc; class rep e l date;
model stoto = e||date;
random rep rep(e l);
repeated date/sub=rep(e l) type=cs;
lsmeans e||date/pdiff;
run;
Proc mixed; by loc; class rep e l date;
model saphids = e||date;
random rep rep(e l);
repeated date/sub=rep(e l) type=cs;
lsmeans e||date/pdiff;
run;
Data three;
infile 'G:\2004 sa.csv' dsd firstobs=2 missover;
input Loc Sam Spray$ Infest$ Date Rep TA TY TotT OA OY TotO Aphids Other;
stott=sqrt (tott+1);
stoto=sqrt (toto+1);
saphids=sqrt (aphids+1);
e=1; l=1;
if spray='Early' then do;l=0; e=1; end;
if spray='Late' then do; l=1; e=0; end;
if spray='No' then do; l=0; e=0; end;
Proc print; proc sort; by loc;
Proc mixed; by loc; class rep e l date;
model stott = e||date;
random rep rep(e l);
repeated date/sub=rep(e l) type=cs;
lsmeans e||date/pdiff;
run;
Proc mixed; by loc; class rep e l date;
model stoto = e||date;
random rep rep(e l);
repeated date/sub=rep(e l) type=cs;
lsmeans e||date/pdiff;
run;
Proc mixed; by loc; class rep e l date;
model saphids = e||date;

```

```
random rep rep(e l);  
repeated date/sub=rep(e l) type=cs;  
lsmeans e||date/pdiff;  
run;
```